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TWO BLUE-STAINING FUNGI ASSOCIATED WITH BARK-BEETLE INFESTATION OF PINES[1]

By CAROLINE T. RUMBOLD [2]

Associate Pathologist, Division of Forest Pathology,[3] Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The relation between bark beetles and blue-stain fungi has become a matter of economic interest in the United States. The insects annually infest many trees, and after their attack a blue stain rapidly develops in the sapwood. Such infestation results in the death of the trees, usually within a short time, and it is probable that the blue stain contributes to their death. Even though the trees may be felled soon after the insect attack, the stained sapwood reduces the commercial value of the lumber cut from them.

The present investigation was undertaken to determine what fungi are associated with bark-beetle damage. Two apparently important fungi have been found in such association. One, new to science, occurs regularly with species of Ips; while another, not previously known in this association in North America, is particularly associated with members of the very destructive genus Dendroctonus. The present paper considers the antecedents and characteristics of these fungi.

In the spring of 1926, F. C. Craighead sent some specimens of shortleaf pine to the Forest Products Laboratory at Madison, Wis. They had been collected in a forest near Asheville, N. C., which had suffered from a severe drought during 1925 and 1926, all the trees showing drought injury and some of them dying. Many of the pines were infested with the bark beetles Dendroctonus and Ips, and the sapwood of these trees was blue stained. Craighead thought that the blue stain spreading from the galleries mined by Dendroctonus was caused by a fungus different from the one staining the wood near the galleries of Ips. Cultures made from specimens sent to the laboratory showed this to be a fact (*13*).[4] Several other blue-staining fungi that appear to be associated with bark-boring beetles were isolated, but for most of them the data collected have been insufficient to make the association certain. Enough evidence has been gathered, however, to indicate such an association for two blue-stain fungi, namely, *Ceratostomella pini* Münch (*C. minor* Hedgc.?) with *Dendroctonus frontalis* Zimm. and *D. brevicomis* Lec., and *C. ips*, n. sp., with *Ips calligraphus* Germ. and *I. grandicollis* Eichh.

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[3] In cooperation with the Forest Products Laboratory, Forest Service, U. S. Department of Agriculture.

[4] Reference is made by number (italic) to Literature Cited, p. 873.

Specimens collected from several parts of the United States furnish the information for the descriptions of these fungi.

These two blue-stain fungi grow from the galleries of bark-boring beetles; the galleries lie within or just under the bark of the tree. These insects, which belong to the family Scolytidae, invade the living trees, usually causing the death of the trees even though the beetles go little deeper than the bark. The beetles of the genus Dendroctonus (*7*) are particularly dangerous to forests, since their attacks often result in the death of even healthily growing trees. The beetles of the genus Ips, although they are capable of killing healthy trees, are more likely to invade those that are injured or sickly.

There is also another group of the Scolytidae with which the study of the blue-stain fungi of the forest brings one into contact. This group is the ambrosia beetles (*8*), the tunnels of which penetrate the sapwood. These beetles propagate certain fungi, upon which they feed, on the walls of their tunnels, and the fungi grow into the wood cells and discolor them, making the holes look black. In time the blue stain spreads through the sapwood. Since the pine-infesting ambrosia beetles invade dying or newly felled trees only, they do not have the economic importance of the true bark borers and bark eaters, but the wood that they have stained often comes to the sawmill.

Some of the specimens sent to the laboratory as infested by the bark beetle Ips had also been invaded by ambrosia beetles (variety not known). The wood surrounding the ambrosia tunnels was likewise blue stained, and the fungus isolated from it was the same as that associated with Ips in the same trees and also found growing in Ips-infested wood that showed no signs of ambrosia beetles.

HISTORY

An association between insect damage and a blue-stain fungus, *Ceratostoma pilifera* Fr., in Germany, was mentioned by Hartig (*4*) as early as 1878. In his description of *C. pini*, Münch (*11*) also mentions the presence of insects in timber stained with this fungus.

In Scotland, MacCallum (*10*) found *Ceratostomella pini* and *C. piceae* Münch staining some standing *Pinus sylvestris* L. where the bark had been riddled by (*Hylesinus*) *Myelophilus piniperda* L. Wilson (*16*) reports a pine in Scotland that was infested with the beetle *Pityogenes bidentatus* Hbst. and showed blue stain near the insect galleries. Although he did not obtain perithecia in the cultures made from the blue-stained wood, he thought the fungus was *C. pini*.

In the United States Von Schrenk (*15*) was the first to show a connection between the infestation of bark beetles and blue stain. His observations were made on *Pinus ponderosa* Dougl., infested with *Dendroctonus ponderosae* Hopk. The fungus that he isolated in culture was *Ceratostomella pilifera* (Fr.) Wint. The results described in the present paper indicate that possibly *C. pilifera* was a secondary blue stain in the wood that he used for starting his cultures and that the fungus that first stained it had lost vitality by the time the wood reached the laboratory. This possibility is borne out by his statement that he was unable to find the perithecia "on the surface of standing trunks under the bark, although a diligent search has been

made for them at all seasons for two years." He was familiar with the perithecia of *C. pilifera*, since, as he states, he had found them "in lumber piles on blue boards and shingles, where they occur in thousands." Further, he adds (*15, p. 22*) that "The spread of the blue fungus within the wood, through the agency of wood-boring beetles, is an occurrence frequently found in many coniferous woods." Hedgcock (*5*) mentions the occurrence of Ceratostomella in the channels of wood-boring beetles. Craighead (*2*) has noted the association between blue stain and the attacks of the bark beetle Dendroctonus. Nelson and Beal's (*12*) work shows the close association between blue stain and the beetles Dendroctonus and Ips. Nelson inoculated trees with blue-stain fungi that had been isolated from the blued wood of trees attacked by bark beetles and also from the bark beetles themselves. These isolations of the fungi from adult beetles were the first successful ones made. Some of these beetles had been dug out of newly made tunnels showing as yet no signs of stain.

Ambrosia fungi in the United States have not been identified, so far as the writer knows. Smith (*14*) examined some ambrosia collected by Hubbard in the burrows of *Corthylus punctatissimus* Zimm. and thought it might be the mycelial or oidial stage of some Ascomycete. The ambrosia fungus of *Xyleborus pubescens* Zimm. as found in maple, described and illustrated by Hubbard (*8, fig. 12*), may be the conidiophores of either *Ceratostomella pini* or *C. ips*, although the illustration does not represent them exactly. Hubbard also states that the ambrosia fungus of *X. xylographus* Say is colorless when young but is usually stained a greenish-yellow and "sometimes resembles a coating of sublimed sulphur" on the walls of the brood chamber. This description reminds one of the greenish-yellow clumps of conidia, which later are honey colored, that characterize the test-tube cultures of *Leptographium lundbergii* Lagerberg and Melin. Von Schrenk (*15*) mentions that *Ceratostomella* sp. was found spreading from the holes made by the ambrosia beetle *Gnathotrichus occidentalis* Hopk. in a fallen log of *Tsuga heterophylla* (Raf.) Sarg. Doane and Gilliland (*3*) have reported a species of Ceratostomella associated with ambrosia beetles, *G. sulcatus* Lec., that were infesting recently felled Douglas fir. The conidial and perithecial stages were observed in the galleries and the fungus stained the wood.

Ceratostomella pini was described by Münch (*11*) as infecting the sapwood of *Pinus sylvestris;* he thought it the most important blue stain of pine. Münch's description of this fungus is clear and his illustrations are particularly good. He mentioned the presence of mites on the specimens of tree timber brought into the laboratory and the presence of the larva of a fly which, he thought, could spread the blue-stain fungus. His description of the morphology of this fungus fits in essential details the fungus connected with the Dendroctonus bark beetles in the United States. Lagerberg, Lundberg, and Melin (*9*) have found this fungus staining lumber in a lumber yard. So far it has not been found in lumber yards in the United States. The perithecium that they have described and pictured differs somewhat from that of the fungus described in this paper. For example, the presence of bristles at the ostiole of the perithecium is particularly described and pictured in their report, while in the United States the fungus usually has no bristles at the ostiole, although in artificial culture under favorable conditions an occasional bristle (one to several

have been counted at one ostiole) is seen. Such bristles, which are long, slender, hyaline hyphae, appear to agree with Münch's description. The bases of the perithecia are rarely hirsute in nature, but are occasionally so in culture. From what has been observed thus far of the fungus in the United States, it grows naturally beneath the bark of pines. The basal part of the perithecium, always rounded at the bottom, seems to make it unsuited for growing on boards, and the loss of vigor when the fungus is exposed to drying conditions tends to confirm this idea. In England MacCallum (*10*) and Wilson (*16*) found the fungus fruiting in similar positions.[5]

INVESTIGATIONAL WORK AND RESULTS

SELECTION OF MATERIAL

Either the blue-stained specimens of pine described in this paper were gathered by entomologists or the beetles taken with them were identified by an entomologist soon after collection. In the laboratory work an endeavor was made to select specimens that had been cut from recently infested pines while the trees were still alive. Some of the specimens sent in, however, had been cut from trees that had been dead for nearly a year. For such specimens it was possible to record no more than the date of collection or of receipt at the laboratory, with the collector's estimate of the age of insect infestation.

METHODS OF ISOLATION AND INVESTIGATION

Cultures of fungi were started in the laboratory by transferring slivers of blue-stained wood to a nutrient agar. These slivers were gouged out with sterile forceps from different regions in the stained area, extending from the beetle galleries just beneath the bark to the heartwood, when the stain extended that far. In this way it was possible to discover whether one or several fungi were growing in the wood. This method of isolation has been described in a previous paper (*13*).

The fungi under investigation were studied in connection with other blue-staining fungi found on hardwoods and softwoods in the forest, the lumber yard, and the factory. Since a blue-stain fungus produces fruits of various sizes on different species of wood, it seemed best to select for this investigation a nutrient medium that could be duplicated at will; only in this way is an accurate comparison of fungi possible. Malt agar [6] was the medium chosen. Except when otherwise stated, the material used for measurement was taken from cultures grown in test tubes on slanted agar. The cultures were kept where the range of temperature was 15° to 30° C. (59° to 86° F.), with an average of 24° C. (75° F.).

[5] Since this paper was prepared the following article has appeared:
GROSSMAN, H. BEITRÄGE ZUR KENNTNIS DER LEBENSGEMEINSCHAFT ZWISCHEN BORKENKÄFERN UND PILZEN. Zeitschrift für Parasitenkunde 3: 56–102. 1930.
Grossman states that the bark-boring beetle Ips plays an important rôle as carrier of blue stain and related fungi, but that the beetle can live without the aid of blue stains. He also states that the adult *Ips typographus* L. carries yeasts as well as blue-stain spores, but is no more dependent on the yeasts for food than on the blue-stain fungi. He describes new species of blue-stain fungi belonging to the Fungi Imperfecti, which were found associated with the insects. Grossman worked also with *Blastophagus piniperda* L., *Scolytus scolytus* Fabr., and other insects.

[6] The formula for this nutrient medium is 25 gm. malt extract (Trommer's Plain), 15 gm. agar, and 1,000 c. c. water. The acidity is pH 5.5 to 5.6.

CERATOSTOMELLA PINI

Ceratostomella pini Münch was found on the following specimens collected in the regions mentioned:

Pinus echinata Mill., freshly infested with *Dendroctonus frontalis*, was collected in the forest near Asheville, N. C. The 15 strains examined were isolated from these specimens, which were collected between April and November over a period of three years. Three strains were isolated by R. M. Nelson from live adult beetles dug out of the galleries beneath the bark.

Pinus ponderosa, freshly infested with *Dendroctonus brevicomis*, was collected in the forest near North Fork, Calif. These specimens were collected in August, September, and October over a period of three years. One of the fungi was isolated by R. H. Colley from a fresh specimen found in the forest.

Pinus ponderosa, freshly infested with *Dendroctonus brevicomis*, was collected in October in the forest near Coeur d'Alene, Idaho.

Pinus taeda L., freshly infested with unidentified bark-boring beetles, was collected in May near Fordyce, Ark.

Pinus virginiana Mill., infested with Pissodes, Monochamus, and mites, was collected in October near Washington, D. C.

The specimens on which *Ceratostomella pini* was vigorously growing were from trees that had recently been invaded by the bark beetles, and the wood was still moist when it was received at the laboratory. Attempts to cultivate the fungus from trees felled and left for a year in the forest failed, even when the fungus fruits under the bark were distinctly recognizable, showing that the fungus had at one time been growing there. When specimens were collected several weeks after the beetles had invaded the trees, other fungi, such as the blue-stain organisms *C. ips*, *C. pilifera*, *Leptographium lundbergii*, *Cadophora* sp., and *Hormonema* sp., and also molds and rots were isolated from the blue-stained wood, as well as *C. pini*. The older the insect infestation the feebler the growth of *C. pini*.

Not only has *Ceratostomella pini* been shown to be associated with Dendroctonus bark beetles by the repeated isolation of the fungus from trees freshly infested with the beetle, but it has also been isolated from live adults of *Dendroctonus frontalis* (*12*). Nelson dug the beetles out of galleries mined so recently that they showed no blue stain. The fungus has never been reported as found staining the freshly sawed boards in lumber yards in continental United States, though cultures have been made from scores of blue-stained pine boards from such yards in different parts of the country.

An odor that seemed identical with the odor of malic acid came from the moist specimens freshly cut from recently infested trees.

APPEARANCE OF DENDROCTONUS-INFESTED TREES

The Dendroctonus bark beetles will invade a living tree gregariously. They bore through the outer bark to the phloem or to the outer layer of wood and there extend their egg galleries. *Ceratostomella pini* will be found growing from these galleries, commonly in longitudinal streaks. (Fig. 1, A and B.) The fungus covers the wood beneath the bark with a black, granular-looking growth, which makes the wood look as though covered with coal dust. The inner bark also shows this growth, mixed with particles of dried rosin.

Beneath the black streaks the fungus grows in toward the heartwood. Hence the cross section of an infected sapwood branch frequently has the appearance of wedges of stained wood extending from the bark to the center. (Fig. 1, C.)

A closer examination of the blackened areas under the bark shows the surface of the wood to be covered with a network of dark-brown

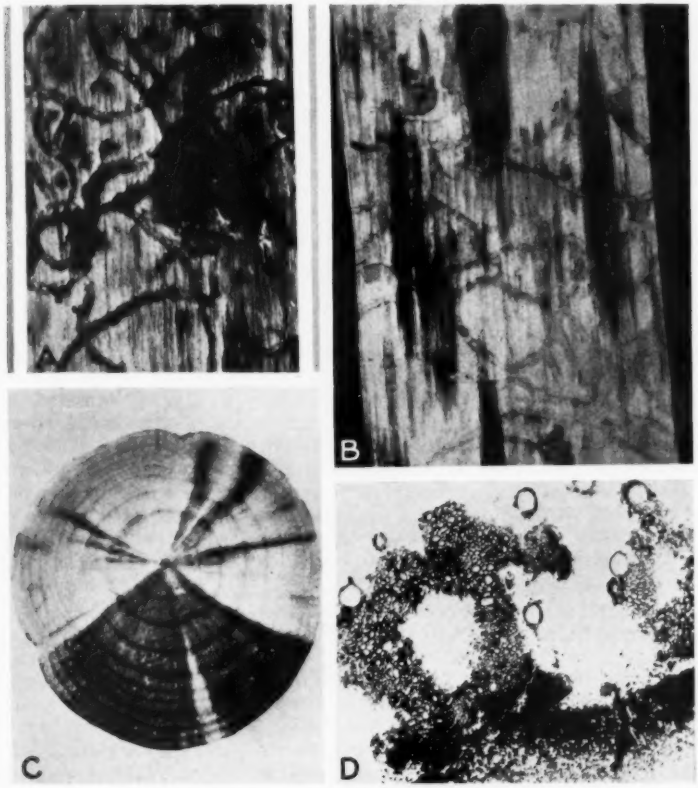

FIGURE 1.—*Ceratostomella pini*. A, Detailed view of *Pinus echinata* with the bark removed, showing galleries of *Dendroctonus frontalis* in the surface and the blue stain caused by the fungus, in some places limited to the frass in the galleries and at others spreading through the wood. B, *Pinus ponderosa* with *D. brevicomis* galleries. The sclerotia make the stained wood appear as if covered with coal dust. C, Cross section of a branch of *P. echinata* showing the stain spreading from the surface to the center. D, Section through two sclerotia and several perithecia, showing the hollowness of the sclerotium and a sclerotioid layer of hyphae growing over the surface of a malt-agar culture. × 28

hyphae. On them are perithecia with short, curved necks and, fewer in number but more easily seen, hard sclerotia of irregular shape many times the size of the perithecia and often having the fruits partially embedded in them. (Fig. 1, D.) Sometimes a sparse, fine, white or gray mycelium surrounds the perithecia. The combination of beetle galleries and spreading fungus with its sclerotia loosen large patches of bark from the tree. In a short time ants,

other insects, and mites are found under the loose bark, and various molds grow over the blue-stain fungus. This partially explains why it is difficult to isolate pure cultures of *Ceratostomella pini* from trees that have been infested for many weeks with the beetles.

Description of the Fungus in Culture

Appearance of Cultures

Cultures of *Ceratostomella pini* were started by transferring slivers of blued wood to malt agar. When these slivers came from a region near the beetle galleries a bacterium and a yeast, apparently always the same organisms, were associated with the fungus. So intimate was the association that the poured plates made from ascospores collected from the perithecia that had developed in the initial cultures showed as many bacterial and yeast as fungus colonies.

When growing on malt agar the bacterium produces an odor somewhat resembling that of acetic acid. In order to separate the fungus from the bacterium the malt agar was acidulated with citric, acetic, or malic acid. The fungus cultures had a darker color when growing on these acid media. The number of vigorously sporulating perithecia increased and the aerial mycelium and conidiophores almost disappeared. Although absence of bacteria may have caused this increased fruiting, possibly the acid reaction of the medium helped. This possibility suggests the further one that on the host tree the bacteria and yeasts may act as a stimulant for the production of perithecia by increasing the acidity of the substratum.

Lagerberg, Lundberg, and Melin (*9*) report that in their cultural work they found that *Ceratostomella piceae* developed perithecia only on a very acid medium.

Some preliminary tests indicate that the eastern strain of *Ceratostomella pini* can withstand the limiting effect of alkali a little better than the California strain.

When first isolated the fungus produces in fresh cultures an odor of malic acid; it is similar to that produced by the fungus growing in wood. The two culture strains of *Ceratostomella pini* found on *Pinus virginiana* without Dendroctonus association lack this malic-acid odor in culture. They also produce a lighter-colored mycelium, more conidiophores, and fewer and slightly smaller perithecia than those associated with Dendroctonus. It is possible that the name *C. pini* can be properly applied only to these strains and that the Ceratostomella associated with Dendroctonus is a distinct species.

Hedgcock's (*5*) *Ceratostomella exigua* from *Pinus virginiana* and his *C. minor* from the galleries of wood beetles on *P. arizonica* Engelm. resemble the writer's cultures from these sources in the size of perithecial base, but the length of neck that he describes is approximately double the diameter of the base of the perithecium and distinguishes them from the writer's material, which has much shorter necks. The sclerotia that are characteristic of *C. pini* are lacking in his descriptions of *C. exigua* and *C. minor*.

When the two are grown in culture the strain associated with *Dendroctonus frontalis* found in the eastern trees differs in appearance from the strain associated with *D. brevicomis* found in the western trees. A mature malt-agar slant culture of the eastern strain isolated

from trees recently infested with *D. frontalis* will show white, gray, and tan mycelium at its top. The center of the slant shows black perithecia embedded in a black mycelium and surrounded with gray aerial mycelium. The individual perithecia can be distinguished by means of the small pearl-colored clumps of ascospores that collect on the tips of the necks of the perithecia and stand out in contrast with the black and gray mycelium. At the base of the slant, wedged between the glass and the agar, sclerotia form. Perithecia generally grow on them. Strands of black mycelium dotted with perithecia grow over the glass.

FIGURE 2.—Typical eastern (A) and western (B) strains of *Ceratostomella pini* in malt-agar cultures of equal age. The lighter color of the eastern strain is due to the greater development of the conidiophores, which obscure the black submerged mycelium. × ½. C, Conidiophores and conidia of *Leptographium lundbergii* in Van Tieghem cell culture, isolated from *Pinus monticola* infested with *Dendroctonus monticolae*. × 180

A mature culture of the western strain when grown under similar conditions is darker in color. There is, usually at the top of the slant, a small amount of gray and tan aerial mycelium, which consists of simple conidiophores, and a rather scant production of conidia. The major portion of the culture appears black, however, and the surface is rough with sclerotia, while black hyphae dotted with perithecia, creeping over the glass, give it the same unkempt appearance that the eastern culture shows. The difference between the two strains can be plainly seen by a comparison of Petri-dish cultures of the same age, growing on the same medium and under similar conditions. (Fig. 2, A and B.) As found in the forest the two strains are alike in appearance, in size, in the shape of the perithecia, and in the method of penetrating the wood—that is, the method of invading the wood rays and of spreading into the tracheids. Cross inoculations, made on steam-sterilized wood in tubes, have shown that the western strain can grow on eastern pines and conversely, and a northern strain on southern pines, provided the moisture content of the wood and the temperature are right.

In the writer's opinion, fungi that are morphologically similar but are not known to be confined to a particular host had best be called by the same name even though the strains show minor differences, as in vigor, size of conidiophores, and quantity of conidia produced.

TABLE 1.—*Dimensions (in microns) of perithecia of Ceratostomella pini*

GROWN ON MALT AGAR

Strains of Ceratostomella pini	Fruits measured	Height of base			Width of base			Length of neck		
		Extreme range	Sextile range [a]	Mean	Extreme range	Sextile range [a]	Mean	Extreme range	Sextile range [a]	Mean
Associated with Dendroctonus frontalis in Pinus echinata, from forest near Asheville, N. C.:										
No. 122	102	52–125	72– 95	84	45–112	63– 88	76	19–136	59– 93	75
No. 123	33	67–126	80–102	92	57–111	71– 94	83	38–132	52– 78	70
No. 134	85	54–150	78–108	92	50–148	74–102	88	43–161	64– 89	78
No. 168	43	50–122	70– 98	83	46–105	66– 91	78	35–122	49– 73	62
No. 187–814	70	60–130	83–107	95	62–129	76–104	89	42–165	58– 86	72
No. 187–838	38	73–138	91–116	105	67–131	78–105	92	47–130	61–115	90
No. 187–841	47	61–112	71– 93	85	53–113	66– 91	79	23–113	45– 74	60
No. 187–842	54	70–122	78–100	92	59–132	73– 97	85	27–131	57– 92	73
No. 202–1	39	82–165	97–138	120	78–163	94–129	113	36–120	54– 83	70
No. 202–2	63	68–130	80–110	97	60–123	72–107	91	21–123	46– 80	63
No. 202–3	28	87–135	97–113	107	84–127	97–113	105	29– 93	50– 72	61
No. 202–4	14	98–124	109–120	116	79–127	97–121	105	42– 80	52– 68	59
No. 202–9	7	82– 99	87– 97	92	77– 93	84– 93	88	60– 80	62– 77	68
Total, range, or mean	623	50–165	80–106	94	45–163	74–101	88	19–165	55– 86	71
From live adult beetles, D. frontalis, from forest near Asheville, N. C.:										
No. 187–89	71	59–180	88–128	105	59–150	79–117	96	27–137	40–107	70
No. 187–811	56	60–160	85–125	106	60–145	85–125	102	35–145	55–125	88
No. 187–813	72	75–168	91–108	104	73–148	82–110	96	43–140	70–112	92
Total, range, or mean for strains associated with adult beetles, D. frontalis	199	59–180	88–120	105	59–150	82–117	98	27–145	55–114	83
Total, range, or mean for all strains associated with D. frontalis	822	50–180	82–109	97	45–163	76–105	90	19–165	55– 93	74
Associated with D. brevicomis in P. ponderosa:										
From forest near North Fork, Calif.—										
No. 165	70	67–136	81–110	97	57–121	79–101	89	35–123	63– 90	76
No. 181	35	60–133	75–111	91	55–128	68 103	84	48 107	59– 80	71
No. 212	71	62–148	85–115	100	58–123	77–100	89	40–100	52– 82	66
From forest near Coeur d'Alene, Idaho, No. 203–939	51	84–150	90–119	104	80–133	90–116	102	35– 97	45– 72	59
Total, range, or mean	227	60–150	83–114	99	55–133	79–104	91	35–123	55– 82	68
Associated with Pissodes, Ips, mites, and Monochamus, in Pinus virginiana, Washington, D. C.: No. 205	105	48–152	81–108	94	44–147	72– 98	86	28–107	45– 70	59
Associated with Monochamus (and also mechanical injuries) in P. virginiana, Washington, D. C.: No. 206	149	67–187	80–112	96	62–163	76–110	92	21– 98	46– 71	60
Total, range, or mean for strains associated with Monochamus, etc.	254	48–187	80–110	95	44–163	74–105	90	21–107	46–71	60
Total, range, or mean for all strains grown on malt agar	1,303	48–187	82–110	97	44–163	76–105	90	19–165	53– 87	70

GROWN ON WOOD

Associated with Dendroctonus brevicomis in Pinus ponderosa, from forest near Coeur d'Alene, Idaho: No. 203–939 [b]	39	64–140	87–110	97	58–120	72– 98	86	24– 98	36– 60	54
Associated with D. frontalis in P. echinata, from forest near Asheville, N. C.:										
No. 122 [b]	48	63–110	72– 96	83	55–102	68– 85	76	19– 79	28– 50	40
No. 123 [c]	142	56–122	72– 96	87	50–116	68– 89	79	25–110	47– 68	60
Total, range, or mean for strains grown on wood	229	56–140	75– 98	88	50–120	69– 90	80	19–110	41– 63	55

[a] The sextile range discards the sixth of the measurements at each end of the extreme range, permitting an approximation of the frequency distribution.
[b] Natural growth as found on tree.
[c] Grown on *Pinus taeda* in culture.

MYCELIUM AND SCLEROTIA

The young mycelium growing in culture from an ascospore or a conidium forms a round, white mat, which spreads over the medium. The margin of the colony is slightly wavy. The young hyphae, which measure 2.3μ to 5μ in diameter, form strands and change color quickly; a culture 4 days old, grown at about 25° C., will look gray and black in the center, and perithecia and sclerotia appear. The dark color is caused by the hyphae changing to a brown color and the cell walls thickening. A dark, granular exudation appears on the walls of some of the old cells, and an old culture appears black. Thin strands of mycelium and single hyphae permeate the agar for short distances.

There is a difference in the vigor of the eastern and the western hyphae in their growth beneath the surface of the malt agar, where the oxygen content presumably is small. The western strain, on entering the medium, produces conidia and perithecia that, as the culture ages, exude ascospores, and the mycelium darkens as it ages and gives the agar a black color. The eastern strain associated with *Dendroctonus frontalis* also enters the agar, but not so vigorously. The hyphae usually are hyaline and a few conidia develop. Some hyphae were brown in color, and some immature perithecia were observed, but no mature fruits. Many of the cells had collapsed or were plasmolyzed, and the phenomenon of small hyphae growing inside of larger ones was seen. The strain isolated from a specimen of *Pinus virginiana* infested with several kinds of insects penetrated the agar slightly. Single hyaline hyphae were seen, the cells of many of which had collapsed. Neither conidia nor perithecia developed below the surface of the agar.

As the culture ages, some of the hyphae become as large as 18μ to 20μ in diameter; these are usually found in or near sclerotia. The sclerotium is formed of hyphae that at first are hyaline but quickly turn brown. The continued increase in diameter of the irregular clump of mycelium puts a strain on it that pulls the central cells apart, thus forming a hole. (Fig. 1, D.) The sclerotia vary in size from those only as large as the perithecia (Table 1) to some measuring 1.5 mm. in diameter. As the culture ages, the surface mycelium develops a sclerotioid tissue.

CONIDIA AND CONIDIOPHORES

At room temperature (about 25° C.) the first new conidia appear about 24 hours after the germination of an ascospore or a conidium. They grow directly from the hyphae. Both the hyphae and the conidia are hyaline. Later on conidiophores appear—simple hyphae growing erect from those spreading over the agar. (Fig. 3.) Single conidia grow from their tips, while others grow out at the side. (Fig. 3, A and B.) Their number quickly increases so that they become bunched like grapes. (Fig. 3, C.) The conidia are larger than the ascopores, ranging from 2.3μ to 6μ in length and 1.2μ to 3μ in width, and are egg-shaped or club-shaped, usually tapering at the attached end. They are pushed off from the conidiophores, but are likely to stick to the other conidia. The conidiophores grow longer, branch, and their bases gradually turn brown, while the slender tips with the conidia remain hyaline. (Fig. 3, D.) The bushy appear-

ance of the conidiophores is very different from that of the conidial strands produced by the common blue-stain fungus of the lumber yard, *Ceratostomella pilifera*. The conidiophores give a gray appearance to the culture, but they disappear as the culture ages. The conidia-producing period is a relatively short one in the life of the fungus, and is shorter for the western strain than for the eastern.

PERITHECIA

Beneath the outer bark of the tree the perithecia develop on the surface of the inner bark or on the wood in which the mycelium is growing. Many of them are partially embedded in the sclerotia. In this situation the base of the perithecium is bare of hyphae except those attaching it to the substratum. When in culture the base occasionally has a few septate hyphae which, growing out into the air, look like hairs. They are usually short, the longest being about one-half the diameter of the base. The base is semiglobular, being taller than wide. (Fig. 4, A.) The ostiole is at the end of a short, curved neck, formed of 12 to 14 hyphae, that tapers slightly toward its tip. Occasionally long, slender, hyaline bristles are seen on the tips; they vary in number from one to several and range in length from 12μ to 37μ. When a perithecium is mature it often looks frayed, the passage of the ascospores having torn the hyphae apart. (Fig. 4, C.) To the eye the perithecium is black, but under the microscope it appears dark brown to black. The tip of the neck is a light brown.

When a Van Tieghem cell culture is about 4 days old perithecia begin to develop. They are formed by the crossing of two hyphae, with a resulting knotting caused by repeated anastomosis of the

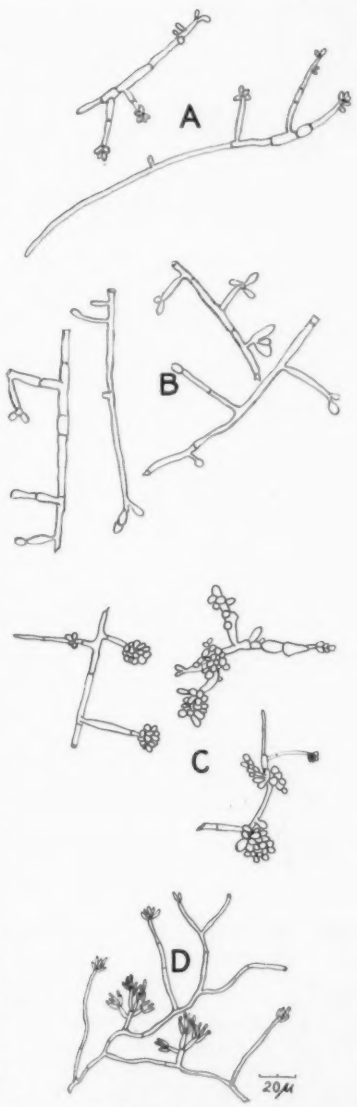

FIGURE 3.—A, Camera-lucida drawing of mycelium and conidia of a *Ceratostomella pini* strain from Idaho, 3 days old; B, an eastern strain of *C. pini* from a Van Tieghem cell culture 4 days old, showing young hyphae and conidia; C, mycelium and conidia, 5 days old, of a *C. pini* strain from California; D, mycelium and branching conidiophores in a Van Tieghem cell culture 12 days old, the same strain as A. (The scale of D applies to all)

hyphae. The young fruit is light brown and has no neck until it is several days old. The formation of asci appears to keep pace with the growth of the neck. The cavity in the base of the immature perithecium is filled with large hyaline cells. The next stage of development in the interior that has been observed is the growth of fertile hyphae from the basal cells of the cavity, between the hyaline cells. (Fig. 5.) If the bases of perithecia that are nearly mature are crushed, they exude globules of an oil-like substance besides the ascospores. The perithecia usually ripen in three weeks,

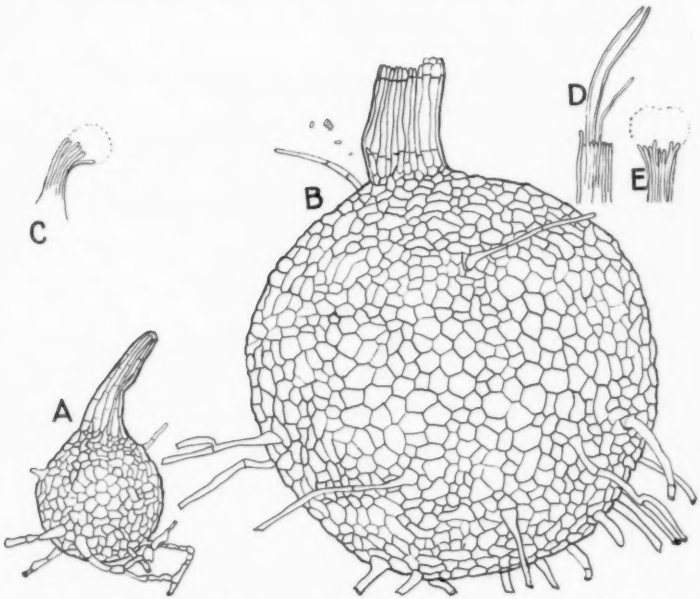

FIGURE 4.—A, Perithecium of *Ceratostomella pini* (× about 270); B, perithecium of *C. ips* with the neck lacking (× about 270); C, hyphae at an ostiole of *C. pini* torn apart by the passage and clumping of ascospores (× about 270); D, ostiole of *C. ips* showing bristles (× about 135); E, ostiole of *C. ips* showing hyphae torn apart by the passage of ascospores (× about 135)

and when ripe they frequently exude masses of spores larger than the fruits themselves.

The fruits, the spores, and the conidia were mounted for study in the glycerin-jelly medium described by Colley (*1*). The fruits were selected from the top, the middle, and the base of the malt-agar slants. Generally those selected from the middle were the largest, but in some instances a strain developed its largest fruits from the drier top or the moister bottom of the slants. In order to allow for the effect of environment on size and shape, all averages were based on the combined measurements of all the fruits from the three positions in the slants, the mean for each position thus being weighted by the number of fruits from it that were measured. Although the relative number taken from the different positions varied greatly,

it was found that these weighted averages did not differ materially from those obtained for comparison of the two methods of averaging, in which all three positions were given equal weight. Table 1 records the final results of the measurements, and Table 2 summarizes them.

The perithecia grown in agar culture were larger than those grown under natural conditions and on wood cultures. The differences, however, fall easily within the range of normal variation. There is no important difference in the size of the perithecia of *Ceratostomella pini* growing on the Atlantic coast and of those growing on the

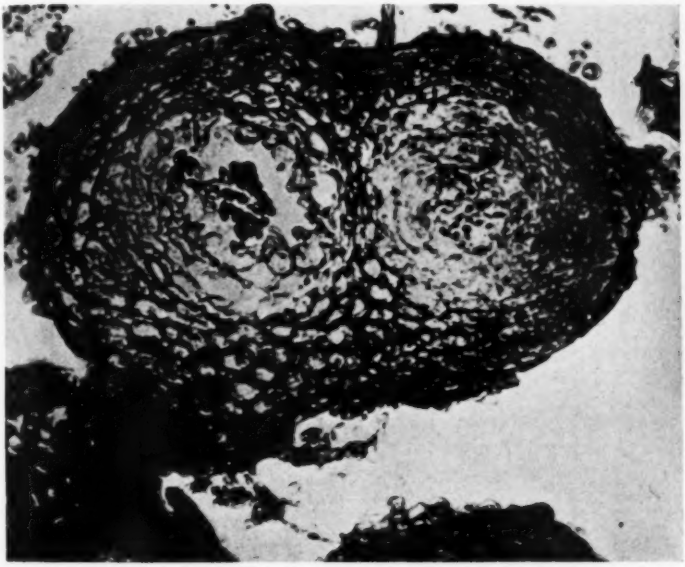

FIGURE 5.—Section of two immature perithecia of *Ceratostomella pini*. The cavity on the right is filled with hyaline cells having large nuclei; the section does not pass through the center of the perithecium. On the left is a section showing a later stage of development of the ascogenic hyphae. × 310

Pacific coast, even though the beetles carrying the fungus are different. (Table 2.)

ASCI

So far only immature asci have been seen. In order to see them, young perithecia were embedded in paraffin, sectioned, and mounted on slides. The asci were then found to be crowded so closely in the interior of the base as to form polyhedrons. Before the ascospores are ejected, however, the walls of the asci disintegrate. In consequence mature perithecia that have been sectioned have shown the cavity crowded with ascospores. The disintegrated asci walls appear to be connected with a mucilaginous substance found with the spores in the perithecium. Sections of perithecia will occasionally show a substance surrounding the ascospores that in the permanent-mount sections has a granular appearance.

TABLE 2.—*Summary of dimensions (in microns) of perithecia of Ceratostomella pini grown on malt agar*

Dimension measured and associated insect	Source		Number of strains having a mean [a] dimension of—													Grand mean dimension
	Host	Region	60μ	65μ	70μ	75μ	80μ	85μ	90μ	95μ	100μ	105μ	110μ	115μ	120μ	
Base height:																μ
Dendroctonus frontalis	P. echinata	North Carolina						3	4	2		2		1	1	94
Do	Live beetles from P. echinata	do										3				105
Pissodes, Ips, Monochamus, and mites	P. virginiana	Washington, D. C								1						94
Monochamus (and also mechanical injury)	do	do								1						96
Dendroctonus brevicomis	P. ponderosa	California and Idaho							1	1	1	1				99
Base width:																
Dentroctonus frontalis	P. echinata	North Carolina				1	2	2	5			2		1		88
Do	Live beetles from P. echinata	do								2	1					98
Pissodes, Ips, Monochamus, and mites	P. virginiana	Washington, D. C						1								86
Monochamus (and also mechanical injury)	do	do							1							92
Dendroctonus brevicomis	P. ponderosa	California and Idaho						1	2		1					91
Neck length:																
Dendroctonus frontalis	P. echinata	North Carolina	4	1	4	2	1		1							71
Do	Live beetles from P. echinata	do			1				2							83
Pissodes, Ips, Monochamus, and mites	P. virginiana	Washington, D. C	1													59
Monochamus (and also mechanical injury)	do	do	1													60
Dendroctonus brevicomis	P. ponderosa	California and Idaho	1	1	1	1										68

[a] The round numbers (microns) of the column headings are the mid-points of the groups into which the actual means fall.

ASCOSPORES

The ascospores when ejected are hyaline. They are cylindrical in shape, although when free from pressure they are slightly curved and the ends round out. They are covered with a thin coating of a mucilaginous substance that does not dissolve readily in water or alcohol. They stick together when ejected and gather in a clump on the neck of the perithecium. The glistening clumps of ascospores are more conspicuous in a culture than the perithecia.

The mean length of the ascospore is 4μ, with an extreme range of 3μ to 5.6μ and a sextile range [7] of 3.6μ to 4.4μ; the mean width is 1.7μ, with an extreme range of 1μ to 3.2μ and a sextile range of 1.4μ to 2μ. These figures are based on the measurements of spores from 4 strains, 2 from North Carolina, 1 from California, and 1 from Idaho.

The ascospores do not germinate in sterile distilled water, but in a malt solution or on malt agar or gelatin they germinate within 24 hours. They swell before producing a germination tube; such tubes may appear on one or on both ends. The percentage of germination on malt gelatin in Van Tieghem cell cultures was about 50.

APPEARANCE OF STAINED WOOD

Wood stained with *Ceratostomella pini* has a neutral gray color shading from light to dark.[8] The wood rays and the resin ducts appear black. When the stained wood has been freshly split open and is still moist, it often has a steel-blue or a purplish-gray cast, one of the reasons why the name blue stain is applied to this discoloration.

A microscopic examination of the wood shows that the fungus first invades the parenchyma cells of the wood rays. The hyphae pass from cell to cell through the pits. As their number increases the end walls of the ray cells are pushed out and later even the side walls are broken out by twisted strands of brown mycelium, which now fill the whole ray. The hyphae in the rays are sometimes very coarse and irregular in shape and in diameter. From the brown mycelium in the rays, hyphae branch off into the neighboring tracheids and resin ducts, using the bordered pits in passing from cell to cell. The openings in the pits are sometimes enlarged by the passage of the growing hyphae through them. Bordered pits filled with the coiled hyphae of *Ceratostomella pini* have been described and pictured by Lagerberg, Lundberg, and Melin (*9*); this condition has been seen occasionally by the writer in heavily infected spring wood. The branching hyphae at first are hyaline, but turn brown as they age. The old hyphae show occasional cells, the walls of which have been roughened by a brown exudation.

Observation has shown that when the hyphae penetrate the ray parenchyma cells the normal contents of the cells disintegrate and disappear except for occasional fine, dark-brown granules. The white color of the walls of the parenchyma cells then changes to a light brown. Apparently the decomposition products of these cells give the stain to the cell walls, and not the hyphae themselves. The long parenchyma cells tend to collapse in the middle, while the end walls still are in contact with each other. The Swedish writers have

[7] The sextile range is obtained by discarding the sixth of the measurements that are smallest and the sixth that are largest and reporting the smallest and the largest values remaining.

[8] RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C., 1912.

described these cells as appearing like hourglasses. Hyphae branch into the neighboring unstained tracheids from the mycelial strands that fill the rays, and since these tracheids are often filled with brown mycelium, it appears to be the lack of cell contents that has kept their walls white.

Sections of wood will sometimes show rays and resin ducts soaked with resin; cells that contain blue-stain hyphae are found in such sections.

The preceding observations are based on wood stained with the strains of *Ceratostomella pini* associated with *Dendroctonus frontalis* in North Carolina and *D. brevicomis* in California and in Idaho. They appear to apply also to the strain of blue stain, found near Washington, D. C., that is associated with some insects but not with Dendroctonus. In the case of this last strain, however, the wood specimens studied were infected with other fungi as well as with Ceratostomella.

Other Fungi Associated with the Dendroctonus Beetle

Two other fungi have been isolated in pure cultures from blue-stained pine infested with the Dendroctonus bark beetle. Brief descriptions of these fungi follow.

LEPTOGRAPHIUM LUNDBERGII

The fungus *Leptographium lundbergii* Lagerberg and Melin, was found on specimens of *Pinus monticola* D. Don., infested with *Dendroctonus monticolae* Hopk., which were collected in the forest near Coeur d'Alene, Idaho. The specimens showed loosened bark with a dark-gray stain on the wood near the beetle galleries, but the black granular growth like that caused by *Ceratostomella pini* was lacking. This stain is caused by a gray and black mycelium growing over the wood. In sheltered places beneath the bark the long, dark conidiophores were visible. The stain spreads in toward the heartwood. A cross section of a branch shows broad wedges of a stain that shades from a light olive gray to an olive gray, while the rays stand out black. The slant test-tube culture developed the black mycelium and the masses of lemon-yellow conidia described by Melin (*9*). Figure 2, C, shows a conidiophore in a Van Tieghem cell culture.

This fungus was isolated also from a section of a girdled tree, which came from the forest near Asheville. The specimen showed a gray-black stain under the bark, and a gray stain had spread evenly through the sapwood. The rays were black.

ATRACTINA SP.

The fungus *Atractina* sp., the conidiophores and septate hyaline conidia of which appear to agree with Von Höhnel's description (*6*), was isolated from a specimen of *Pinus ponderosa* that had been infested with *Dendroctonus ponderosa* Hopk. the previous year. The specimen, which was collected in the Kaibab National Forest, Ariz., showed the primary egg galleries beneath the bark with a black stain around them, and the sapwood had a gray stain with black rays extending to the heartwood. Although the shapes of the conidiophores sometimes resemble those of *Leptographium lundbergii*, the color of the massed conidia is white. The color of the cultures, black and gray, easily separates this fungus from Leptographium with its lemon-yellow masses of conidia.

TABLE 3.—*Dimensions (in microns) of perithecia of Ceratostomella ips*

GROWN ON MALT AGAR

Strains of Ceratostomella ips	Fruits measured	Height of base			Width of base			Length of neck		
		Extreme range	Sextile range [a]	Mean	Extreme range	Sextile range [a]	Mean	Extreme range	Sextile range [a]	Mean
Associated with Ips calligraphus in Pinus echinatus, from forest near Asheville, N. C.:										
No. 170	67	98–281	144–224	178	91–253	130–205	164	370–1, 411	52b–1, 190	796
No. 171	19	96–270	121–175	158	55–222	108–172	140	328–1, 231	384– 963	697
No. 202–13	24	150–320	180–220	214	150–300	180–210	209	1, 075–3, 110	1, 460–2, 020	1, 838
No. 202–14	32	150–300	200–240	213	150–260	180–230	204	775–2, 840	1, 355–2, 240	1, 790
Associated with I. calligraphus in P. sylvestris, from forest near Tuckerton, N. J.: No. 191	30	120–290	202–232	221	120–280	203–232	220	435–2, 640	1, 292–1, 710	1, 555
Associated with I. calligraphus in P. rigida near Rising Sun, Md.: No. 211	23	160–250	190–225	209	150–240	195–220	205	635–1, 890	1, 010–2, 520	1, 282
Total, range, or mean for strains associated with I. calligraphus	195	96–320	170–223	196	55–300	161–212	187	328–3, 110	939–1, 561	1, 252
Associated with I. grandicollis in P. echinata, from forest near Asheville, N. C.:										
No. 187–84	22	160–267	192–240	213	162–248	195–235	213	613–1, 350	850–1, 212	995
No. 202–11	31	140–230	167–215	193	140–230	165–210	190	620–2, 755	1, 170–1, 905	1, 538
From live adult beetles, I. grandicollis, from forest near Asheville, N. C.: No. 187–S22	29	130–240	150–220	183	120–250	140–210	179	215–2, 290	460–1, 470	976
Total, range, or mean for strains associated with I. grandicollis	82	130–267	168–223	195	120–250	164–217	192	215–2, 755	833–1, 565	1, 194
Associated with Ips sp. in P. resinosa from forest near Petersburg, Pa.:										
No. 136	75	115–300	195–266	230	134–301	179–258	219	635–2, 668	1, 025–1, 990	1, 472
No. 137	40	158–273	192–238	219	152–273	172–224	203	715–2, 318	1, 112–1, 812	1, 456
No. 139	44	148–270	191–252	219	150–290	188–243	213	478–2, 090	840–1, 812	1, 362
Associated with Ips sp. in P. palustris, from forest near Starke, Fla.:										
No. 157	64	125–300	171–232	206	120–293	163–237	201	470–3, 860	775–1, 300	1, 141
No. 158	14	123–230	172–220	189	113–228	152–208	179	375–1, 371	458–1, 095	770
Total, fruits measured for strains associated with Ips sp	237									
Total, range, or mean for all strains grown on malt agar	514	96–320	177–234	206	55–301	167–226	198	215–3, 860	906–1, 620	1, 273

GROWN ON WOOD

Associated with Ips calligraphus in Pinus echinata, from forest near Asheville, N. C.: No. 202–12 [b]	6	160–240	180–230	198	180–230	190–220	200	400–1, 010	690– 950	802

[a] The sextile range discards the sixth of the measurements at each end of the extreme range, permitting an approximation of the frequency distribution.
[b] Natural growth as found on tree.

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CERATOSTOMELLA IPS, N. SP.

Ceratostomella ips, n. sp., was found on specimens of the following species collected in the regions named:

Pinus echinata, freshly infested with *Ips calligraphus* Germ., in the forest near Asheville, N. C., was collected in June, August, and October over a period of three years. Four strains, reported in Table 3, were isolated from the specimens, and No. 202–12, a natural growth in the forest, also came from this region. One of the specimens showed *Ceratostomella pini* fruiting under the bark as well as *C. ips*, and cultures of both fungi were obtained from the blued wood.

Pinus rigida Mill., a dead tree infested with *Ips calligraphus*, was collected in October near Rising Sun, Md. It showed *Ceratostomella pini* and *C. pilifera* fruiting under the bark as well as *C. ips*. Although the majority of the cultures developed *C. ips*, some developed *C. pini*. Molds were present in the cultures.

Pinus sylvestris L., freshly infested with *Ips calligraphus*, was collected in June in the forest near Tuckerton, N. J.

Pinus echinata, freshly infested with *Ips grandicollis* Eichh., was collected in May, June, and October, over a period of two years, in the forest near Asheville, N. C. The three cultures reported in Table 3 were isolated from these specimens. Two of them were isolated by Nelson, the first from stained wood and the second from live beetles dug out of insect galleries.

The fungus has also been isolated from the following pines, infested with Ips (the species of Ips is not known):

Pinus resinosa Soland., freshly infested with *Ips* sp., was collected in June in the forest near Petersburg, Pa. The specimens were collected in a plantation of young trees, in good condition in other respects, in which heavy mortality was connected with the attack of the insect and the fungus. One of the specimens came from a tree showing older infestation; the blue-stain fungi *Graphium rigidum* (Pers.) Sacc. and *Leptographium lundbergii* developed from it.

Pinus palustris Mill., freshly infested with *Ips* sp., was collected in September in the forest near Starke, Fla.

Pinus palustris, dead timber infested with unidentified insects, was collected in March near Bogalusa, La. Nematodes were also present. A brown fungus, which may be a Teichosporella and a Trichoderma, was cultured from the blued wood as well as *Ceratostomella ips*.

The trees from which *C. ips* was isolated in pure culture had been invaded recently by the bark beetle Ips, and the wood was fresh when received at the laboratory. Those trees that had stood for some time after infestation showed secondary infection. *C. ips* has not been found staining freshly sawed lumber in the lumber yard.

In the summer of 1931 *Ceratostomella ips* was isolated from specimens of *Pinus rigida* cut from trees recently infested with *Ips avulsus* Eichh., near Asheville, N. C. *C. ips* was also isolated from a specimen of *P. strobus* L. infested with *I. pini* Say, near Petersham, Mass.

APPEARANCE OF IPS-INFESTED TREES

The Ips bark beetles bore through the outer bark to the phloem and there extend their galleries. From the galleries beneath the bark, blotches of black stain spread, generally in longitudinal streaks.

(Fig. 6.) The stain produced in the wood by the fungus is a slate gray.

A close examination of the black blotches under the bark shows a network of dark-brown mycelium, and perithecia so large that they

FIGURE 6.—Specimen of *Pinus echinata* with the bark removed, showing galleries of *Ips calligraphus* and the blue stain caused by *Ceratostomella ips*

can be seen with the naked eye. These perithecia usually are growing in the frass in the galleries.

The association between *Ceratostomella ips* and the Ips beetles has been shown by repeated isolation of the fungus from trees freshly infested with the beetles. Nelson[9] has been successful in isolating

[9] Unpublished data.

the fungus also from the live adults of *Ips grandicollis*, which he had dug out of their tunnels in the trees. The two strains isolated from these two sources look alike in their natural situation and in culture. The strain associated with *I. calligraphus* has the same rate of growth as that associated with *I. grandicollis*.

Description of the Fungus in Culture

Appearance of Cultures

In malt agar *Ceratostomella ips* shows a less luxuriant and varied growth than *C. pini*. The young fungus colonies are white, but when about 5 days old the hyphae in the center of a colony begin changing to a warm sepia, although the center will still show branched conidiophores, the bases of which are darkening. The culture then quickly turns a jet black, with a scant aerial mycelium, warm sepia in color, showing here and there on the surface. An old culture is colored olive green, warm sepia, and black. The striking characteristic of this fungus and the one by which it can easily be distinguished is its large perithecia embedded in the nutrient medium, where the amount of oxygen available must be very small. The necks of these fruits point in every direction. Single hyphae or sometimes a simple strand of two or three will be found growing through the agar. Such hyphae remain hyaline for some time, so that at first the perithecia that develop below the surface appear to grow in the agar unattached to the black mat of mycelium covering the surface. As the culture ages and dries these hyphae turn brown.

A bacterium and yeasts were isolated from the slivers of wood that were taken from near the galleries to start the cultures. The malt-agar culture produces an odor that appears to be identical with that of acetic acid.

Mycelium

The hyphae growing from ascospores or conidia are hyaline, septate, and between 2μ and 3μ in diameter. They show a tendency to form loose strands. As the culture ages they increase in size, and their walls turn brown and thicken. Occasional cells show a brown, granular-looking exudate. The mycelium exposed to the air forms a sclerotioid tissue of closely interwoven dark-brown hyphae 4μ to 6μ in diameter, which appears jet black to the eye. As already stated, the hyphae that grow into the medium remain hyaline for so long a time that their presence in the middle of the agar was not suspected until the perithecia developed.

Conidia and Conidiophores

Conidia, which at first form directly on the young hyphae, can be seen about 24 hours after an ascospore or a conidium has germinated. Those formed first are small, sometimes only 2μ long and 1μ wide. They are hyaline and obovoid. Later they form on simple conidiophores in a cluster that increases in number as the fungus ages. The conidiophores branch as they grow older until they look like small bushes. In time the bases of the conidiophores turn brown, but the conidia-bearing tips and the conidia themselves remain hyaline. The later conidia range from 3μ to 10.5μ long and 1μ to 3μ wide. They are usually clavate. Larger ones may be seen in the clusters of conidia

pushed from the conidiophores, but it is possible that these are about to germinate, since conidia swell before producing a germination tube. (Fig. 7.)

The period in which conidia and conidiophores are produced is a short one in the life cycle of *Ceratostomella ips*, and they disappear as the perithecia mature.

PERITHECIA

In culture the perithecia usually develop on the mycelium growing over the surface of the medium, but they also develop in it. Their development and also their orientation seemingly are independent of both light and gravity. When growing beneath the bark of a tree, the perithecia usually have short, thick, and crooked necks. Apparently there is not space enough between the bark and the wood for the development of the long necks that they can produce in cultures.

FIGURE 7.—Drawing of a conidiophore and conidia of *Ceratostomella ips* grown in a Van Tieghem cell culture

The base of the perithecium is globular. The occasional brown and septate hyphae, 5μ to 24μ long, that grow like hairs from the base, are inconspicuous on the mature perithecia and are lacking on many of the bases, which are then bare except for the hyphae connecting the fruit and the vegetative mycelium.

The mature perithecium is black, and the tip of the neck is a warm sepia in color. The neck is made of 18 to 22 brown hyphae, which are septate, and the normal cells are uniform in size in the uninjured necks. (Fig. 4, B.) These hyphae apparently are sensitive to pressure, for most of the necks are irregular in thickness and are crooked because of the irregular growth of the cells. Usually there are no bristles at the ostiole, although occasionally a few are seen, which are irregular both in number and in length, measuring from 27μ to 45μ. (Fig. 4, D.) An old perithecium that has discharged its spores will often show a frayed tip on its neck; such fraying is apparently caused by the tearing apart of the hyphae in the passage of the ascospores. (Fig. 4, E.) Often the ascospores are discharged by means of the bursting of the base of the perithecium, however, and then the neck and the ostiole of course are not used. This phenomenon has been seen on trees and in cultures and is especially common when the perithecia are embedded in the nutrient medium. The perithecia, like those of *Ceratostomella pini*, are formed by the knotting and anastomosis of two or more hyphae that have crossed one another. When grown in culture at room temperature the perithecia are visible in a week after this formation, and in about two weeks more the fruits have matured.

The development of the ascospores in the cavity of the base appears to keep pace with the growth of the neck. The cavity of an immature fruit is first filled with large, hyaline cells, at which time the neck has not formed. These large cells then begin to show a differentiation near the top of the cavity, where the neck is forming. As seen in prepared slides, they take a heavier stain than the hyaline cells, and the nuclei become large and conspicuous. Hyphae, which stain readily, appear. These grow between the large cells and develop the asci on their tips. As the asci form, the large cells disintegrate and the interior becomes filled with an oily substance and asci.

ASCI

Occasionally the asci can be seen when an immature perithecium is crushed, and they are easily seen in the paraffin sections of the perithecia, where they appear crowded in the cavity into irregular, polyhedron shapes. They measure between 7μ by 9μ and 8μ by 10.4μ.

In the early stage the whole ascus takes a heavy stain with safranine, but later the stain collects in the ascospores alone, the ascus wall no longer accepting it. As the ascospores increase in size, there appears in the interior of the cavity a substance that in the permanent-mount sections has a granular appearance and stains faintly with safranine. Streaks of it show between the closely packed ascospores in the ripening perithecium. (Fig. 8.) This may be the oily substance that exudes from young perithecia when they are crushed. The walls of the asci disappear while the asci are still in the cavity.

ASCOSPORES

The ascospores, as a rule, are crowded in the ascus in two rows, and they are often seen pressed together in groups of four. Sometimes they are irregularly arranged, however, for apparently they slide about inside the ascus as they increase in size, and the asci themselves are put under pressure as the cavity becomes crowded. The walls of the asci disappear before the ascospores are discharged. The ascospores are cylindrical in shape while in the cavity and when first discharged. When floating freely in water they are hyaline, slightly curved, and the ends gradually round out. Usually there is an oil globule in each end of the spore. The spores, which are fairly regular in size and shape, have a mean length of 3.8μ, an extreme range of 2.9μ to 4.6μ, and a sextile range of 3.3μ to 4.2μ, while the mean width is 2μ, the extreme range 1.2μ to 2.8μ, and the sextile range 1.9μ to 2.5μ. These measurements of *Ceratostomella ips* are of different strains, two from Pennsylvania and one from Florida.

The spores are covered with a thin film of a mucilaginous substance that causes them to cling together. They are ejected in a thread like a spider's, which clumps and dries in a glistening mass on the neck. This clump can not be knocked off easily, although a moistened needle will readily remove it. On drying, the ascospores shrink to about one-half their original size.

The percentage of germination of the fresh ascospores of *Ceratostomella ips* on malt agar or gelatin is high, about 80 per cent in 24 hours. This percentage is higher than that shown by the spores of the lumber-yard blue stain under the same conditions. The spores swell when about to germinate and produce one or two germination tubes.

Appearance of Stained Wood

The *Ceratostomella ips* stain beneath the bark in the beetle galleries is slate black in color. When the wood is split open a slate-gray or blackish-slate stain may be seen spreading from the tunnels of the beetles toward the heartwood. The wood rays and resin ducts appear black.

When examined with the microscope, freshly cut sections of the stained wood that have not been heated or boiled show the wood rays

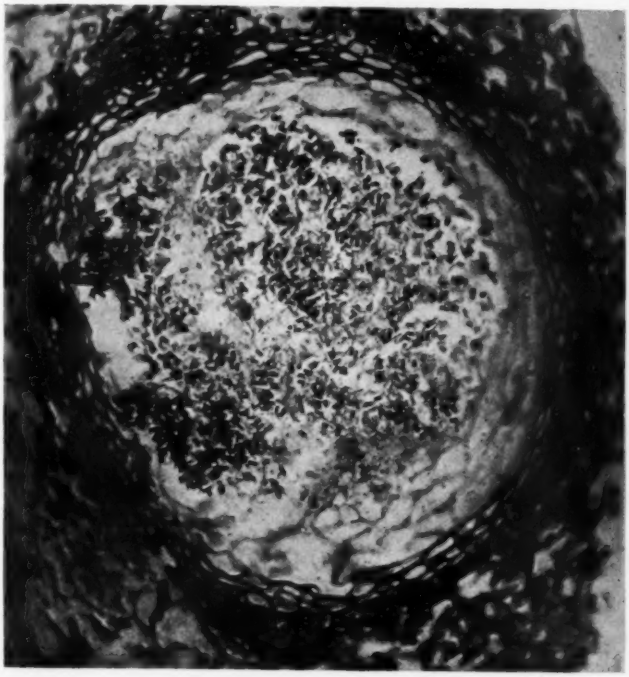

Figure 8.—Section of the base of a perithecium of *Ceratostomella ips* showing an irregular development of asci and ascospores. × 490

filled with brown hyphae growing from cell to cell. When the hyphae penetrate the ray parenchyma cells the normal contents of the cells disintegrate and disappear, only occasionally leaving a brown, granular residue. Seemingly it is the decomposition products of the ray cells that give a light-brown stain to the walls. However, ray cells have been seen in which the yellow cast appears to be due solely to the presence of the brown mycelium. The walls of the tracheids are not stained. The diameter of these hyphae usually is greater than that of the hyphae of *Ceratostomella pini*, which may be a reason for the darker discoloration of wood well infected with *C. ips* when compared with wood infected with *C. pini*. The infected ray cells take on the "hourglass" appearance described for *C. pini*.

As the hyphae in the ray cells increase in number the end walls of these cells are pushed out and later the side walls are broken out, so that the ray is filled with a strand of brown hyphae. The cells of some of the hyphae occupying the rays are large and irregular in shape. From these strands hyphae branch into the neighboring tracheids, commonly using the pits as passageways. The hypha when it first invades a wood cell is hyaline. It appears to brown more slowly than the mycelium of *Ceratostomella pini*, for the cells are often filled with hyphae brown in one part and hyaline in another. This slow change of color may partly explain the fact that less mycelium is seen in these wood cells than in the wood infected with *C. pini*.

The Ips beetle usually infests weakened trees. The cells of these trees may have relatively little food in them, and this may be a reason for the poorer mycelial development of *Ceratostomella ips*.

Technical Description

Ceratostomella ips n. sp.

Young colonies with conidia white changing to sepia and black, with perithecia; young hyphae hyaline, 2μ to 3μ in diameter, old hyphae brown, 3.5μ to 10.4μ in diameter; conidia hyaline appear first on hyphae, obovoid to ellipsoidal, range from 3μ long, 1μ wide, to 10.5μ long, 3μ wide; young conidiophores single hyaline hyphae bearing conidia in clusters, older conidiophores single brown hyphae bearing at their tops branches, biverticillate, on the tips solitary conidia that collect in a head held together by a mucilaginous substance; perithecia nongeotropic, globose, slightly hirsute, height of base, extreme range 96μ to 320μ, sextile range 177μ to 234μ, mean 206μ, width of base, extreme range 55μ to 301μ, sextile range 167μ to 226μ, mean 198μ, length of neck, extreme range 215μ to 3,860μ, sextile range 906μ to 1,620μ, mean 1,273μ, the ostiole usually without terminal filaments—those bristles seen measure 27μ to 45μ long—sometimes ostiole is not used, the ascospores escaping from the broken base; asci ephemeral, polyhedral, 7μ to 8μ by 9μ to 10.4μ; ascospores 8, hyaline, cylindrical or slightly curved, 2.9μ to 4.6μ long, sextile range 3.3μ to 4.2μ, mean 3.8μ, and 1.2μ to 2.8μ wide, sextile range 1.9μ to 2.5μ, mean 2μ.

On sapwood of *Pinus echinata* infested with *Ips calligraphus* near Asheville, N. C.; *P. rigidia* infested with *I. calligraphus* near Tuckerton, N. J.; *P. echinata* infested with *I. grandicollis* near Asheville, N. C.; *P. resinosa* infested with *Ips* sp. near Petersburg, Pa.; *P. palustris* infested with *Ips* sp. near Starke, Fla.

Leptographium lundbergii, Associated with the Ips Beetle

Leptographium lundbergii, which has been mentioned in connection with Dendroctonus, has been isolated from specimens of the following species collected in the regions named:

Pinus echinata, infested with *Ips calligraphus*, was collected in June in the forest near Asheville, N. C.

Pinus resinosa, infested with *Ips* sp., was collected in June in the forest near Petersburg, Pa.

To all appearances the fungus from these sources resembles the one isolated from the Dendroctonus-infested trees and a girdled tree.

DISCUSSION

The term "association" as used in this paper in the descriptions of the connection between *Ceratostomella pini* and Dendroctonus and *C. ips* and Ips does not have the significance of the vital association that exists between the ambrosia beetles and the ambrosia fungi. The association appears to be a more casual one for the bark-boring beetles, which are not known to be dependent on fungi

for food. The fact that *C. ips* has never been found except in association with species of Ips and that the vigorously growing strains of *C. pini* have been found in this country only in association with species of Dendroctonus, indicates something specific in the association, but no clue has been obtained as to the basis for this specificity. Further, the conditions when the beetles first invade the trees may be especially favorable to this fungus, and its resulting growth in turn may possibly help the invading beetles to become established. Many fungi were isolated from the blue-stained wood of insect-infested trees, but as a rule only one fungus would be cultured from the stained area of a specimen collected when the tree had just been invaded by bark borers.

Investigation of trees growing in other regions and infested with other varieties of Dendroctonus and Ips may show whether these two blue-staining fungi are vital factors in the life cycle of the Dendroctonus and the Ips bark beetles.

The vigor of growth in culture of the *Ceratostomella pini* that is associated with *Dendroctonus frontalis* and *D. brevicomis* appears to have been affected by environment. The strain associated with *D. brevicomis* in the western forests grows more vigorously than the one associated with *D. frontalis* in the eastern forests. The third strain, morphologically similar, which has been isolated from pines near Washington, D. C., that were infested with insects but not with Dendroctonus, is feeble in growth in comparison with the other two strains. Possibly, as has been suggested before, this third strain is the European *C. pini*. It should not be surprising to find European blue stains in the eastern part of the United States and American ones in Europe after consideration of the mass inoculation of these regions going on in the continuous commercial exchange of products packed in boxes made of blue-stained wood. *C. pini* in Europe has not been reported intimately associated with insects. This independence of association might make it able to survive the ocean journey; the fungi associated with bark beetles in the forest apparently are unable to infect the freshly sawed lumber in lumber yards. Laboratory tests showing the response of the three strains to acidity, temperature, moisture, and food variations may clarify this problem.

Ceratostomella ips has been found growing from the galleries made by *Ips calligraphus*, *I. grandicollis*, and *Ips* sp. in pines of the Eastern States. Whether it is associated with other varieties of Ips is not known.

SUMMARY

The bark-boring beetle Dendroctonus infests growing pines. The sapwood of infested pines becomes blue stained soon after the beetles enter the trees. The trees usually die shortly afterwards.

The blue-stain fungus growing from the galleries of *Dendroctonus frontalis* in eastern pines and from those of *D. brevicomis* in pines of California and Idaho has been identified as *Ceratostomella pini*. The same species has been found near Washington, D. C., staining pines that had been injured mechanically and were infested with insects other than Dendroctonus and with mites.

In artificial culture the strain of *Ceratostomella pini* associated with *Dendroctonus brevicomis* grows and fruits most vigorously, that asso-

ciated with *D. frontalis* is next in vigor, and the strain not associated with Dendroctonus is the feeblest.

Ceratostomella pini can be easily recognized when it is growing beneath the bark of Dendroctonus-infested trees. Its black sclerotia and perithecia give a black and granulated appearance to the phloem of the trees, so that the phloem appears as though covered with coal dust.

The stain caused by *Ceratostomella pini* grows from the beetle galleries toward the heartwood. The color of the stained wood is gray, shading from a light neutral to a dark neutral gray. The rays and resin ducts appear black. When the stained wood has been freshly split open and is still moist it has a steel-blue or purplish cast. The brown hyphae of *C. pini* grow first into the parenchyma cells of the wood rays, and spread from them into the adjoining tracheids, generally using the bordered pits as passageways. As the number and the diameter of the hyphae in the cells increase, the end walls of the ray cells are broken out and rays become filled with strands of hyphae.

The bark-boring beetle Ips infests pines that are growing slowly. The attack of this beetle also results in the death of the trees, and the sapwood of infested pines becomes blue stained shortly after the beetles enter the trees.

The blue-stain fungus growing from the galleries of *Ips calligraphus* and *I. grandicollis* in the eastern pines has been found to be a Ceratostomella not previously described. For this species the name *Ceratostomella ips* is suggested. This fungus has been found in Ips-infested pines in an area extending from Pennsylvania to Florida. The strain associated with *I. calligraphus* and the one associated with *I. grandicollis* show no differences in artificial culture.

Ceratostomella ips can be recognized when it is growing beneath the bark of Ips-infested pines. Its perithecia, when growing in the frass of the insect galleries, are often large enough to be seen with the naked eye.

The stain caused by *Ceratostomella ips* grows from the insect galleries toward the heartwood. The color of the stained wood is a slate gray, shading to a slate black. The rays and resin ducts appear black. When it has been split, freshly stained moist sapwood has a steel-blue cast.

The hyphae of *Ceratostomella ips* are hyaline when young and dark brown when old. They grow into the parenchyma cells of the wood rays first, fill them with strands of mycelium, and then spread into the tracheids and the resin ducts, commonly growing through the bordered pits. The perithecia are large and appear black to the naked eye. They apparently are not affected in growth by light or gravity, since in culture the perithecia form in the middle of the nutrient medium as well as on the surface and the necks of the fruits grow in every direction. The necks and their ostioles are not always used in the discharge of the ascospores; in some instances the bases burst and the ascospores ooze out. Perithecia can be seen ejecting their spores in this manner, both in culture and when growing naturally beneath the tree bark.

LITERATURE CITED

(1) COLLEY, R. H.
1925. A BIOMETRIC COMPARISON OF THE UREDINIOSPORES OF CRONARTIUM RIBICOLA AND CRONARTIUM OCCIDENTALE. Jour. Agr. Research 30: 283–291, illus.

(2) CRAIGHEAD, F. C.
1928. INTERRELATION OF TREE-KILLING BARK BEETLES (DENDROCTONUS) AND BLUE STAIN. Jour. Forestry 26: 886–887.

(3) DOANE, R. W., and GILLILAND, O. J.
1929. THREE CALIFORNIA AMBROSIA BEETLES. Jour. Econ. Ent. 22: 915–921.

(4) HARTIG, R.
1878. DIE ZERSETZUNGSERSCHEINUNGEN DES HOLZES DER NADELHOLZBÄUME UND DER EICHE IN FORSTLICHER, BOTANISCHER UND CHEMISCHER RICHTUNG. 151 p., illus. Berlin.

(5) HEDGCOCK, G. G.
1906. STUDIES UPON SOME CHROMOGENIC FUNGI WHICH DISCOLOR WOOD. Missouri Bot. Gard. Ann. Rpt. 17: 59–114, illus.

(6) HÖHNEL F. VON.
1904. ZUR KENNTNIS EINIGER FADENPILZE. Hedwigia 43: [295]–299.

(7) HOPKINS, A. D.
1909. BARKBEETLES OF THE GENUS DENDROCTONUS. U. S. Dept. Agr., Bur. Ent. Bul. 83, 169 p., illus.

(8) HUBBARD, H. G.
1897. THE AMBROSIA BEETLES OF THE UNITED STATES. U. S. Dept. Agr., Div. Ent. Bul. (n. s.) 7: 9–30, illus.

(9) LAGERBERG, T., LUNDBERG, G., and MELIN, E.
1927. BIOLOGICAL AND PRACTICAL RESEARCHES INTO BLUEING IN PINE AND SPRUCE. Svenska Skogsvårdsföreningens Tidskr. 25: [145]–272, [561]–739, illus. [Resumé in Swedish, p. 692–739.]

(10) MACCALLUM, B. D.
1922. SOME WOOD-STAINING FUNGI. Brit. Mycol. Soc. Trans. 7: 231–236, illus.

(11) MÜNCH, E.
1907–8. DIE BLAUFÄULE DES NADELHOLZES. Naturw. Ztschr. Forst u. Landw. 5: 531–573; 6: 32–47, 297–323, illus.

(12) NELSON, R. M., and BEAL, J. A.
1929. EXPERIMENTS WITH BLUESTAIN FUNGI IN SOUTHERN PINES. Phytopathology 19: 1101–1106.

(13) RUMBOLD, C. T.
1929. BLUE-STAINING FUNGI FOUND IN THE UNITED STATES. Phytopathology 19: 597–599.

(14) SMITH, E. F.
1896. AMBROSIA. Amer. Nat. 30: 318–319.

(15) VON SCHRENK, H.
1903. THE "BLUING" AND THE "RED ROT" OF THE WESTERN YELLOW PINE, WITH SPECIAL REFERENCE TO THE BLACK HILLS FOREST RESERVE. U. S. Dept. Agr., Bur. Plant Indus. Bul. 36, 40 p., illus.

(16) WILSON, M.
1922. SEASONAL DISEASES IN THE SCOTS PINE. Brit. Mycol. Soc. Trans. 13: 81–85.

ROOT DEVELOPMENT AS RELATED TO CHARACTER OF GROWTH AND FRUITFULNESS OF THE COTTON PLANT[1]

By FRANK M. EATON

Formerly *Assistant Physiologist, Division of Cotton, Rubber, and Other Tropical Plants, Bureau of Plant Industry, United States Department of Agriculture*[2]

INTRODUCTION

In this paper are reported the results of observations on the influence of the fruiting and branching habits of the cotton plant upon the growth of roots. The experimental plants were grown at the United States Field Station, Sacaton, Ariz., in 1927.

In the course of a study of the reactions of the cotton plant to defloration and defruiting (*5*)[3] it became apparent that changes in root development as marked as the changes in aerial development resulted from these experimental treatments. Although the factors determining the growth dominance of certain plant parts over others are too little understood to permit of adequate causal analyses, it is none the less desirable that the facts in this regard be worked out. This is particularly true in the case of an important crop plant such as cotton, the growth habits and fruitfulness of which are especially responsive to cultural treatments and environmental changes. The cotton plant affords unusually suitable material for investigations, not only of growth correlation but also of translocation of photosynthetic and elemental substances (*9*, *10*, *12*).

Much of the literature dealing with roots and their growth has been reviewed by Weaver (*14*) and by Weaver and Bruner (*15*). Differences in the distribution of roots in the soil have been shown to be characteristic of different species and varieties, and the relative development of the roots and the aerial portions of plants has been studied in relation to soil temperature, fertility, texture, and moisture. Garner and Allard (*7*) report that the length of day is of considerable importance in this regard. The root systems of cotton plants have been traced through the soil mass by Balls (*1*), King (*8*), Collings and Warner (*3*), and others. The differences in root distribution and abundance observed by them were associated with such variables as soil water and fertility.

Identical treatments applied to both Pima Egyptian and Acala upland cotton form the basis for the principal comparisons herein reported. The plants were grown in cans, and the treatments were as follows: (1) Normal fruiting and branching habits of the plants not interfered with; (2) fruiting prevented by the removal of all floral buds; (3) aerial growth confined, by bud removal, to the main stalks and main-stalk leaves. The normal Acala plants were compared also with plants that had been subjected to two different treatments, some having been allowed to wilt severely before each

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[2] Now associate physiologist, Division of Western Irrigation Agriculture, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] Reference is made by number (italic) to Literature Cited, p. 882.

watering and some having been grown with one plant instead of the customary two plants in each pot.

METHODS

The pot-culture methods employed have been previously referred to (*6*). Each of the cans was of a size to hold approximately 110 kgm.

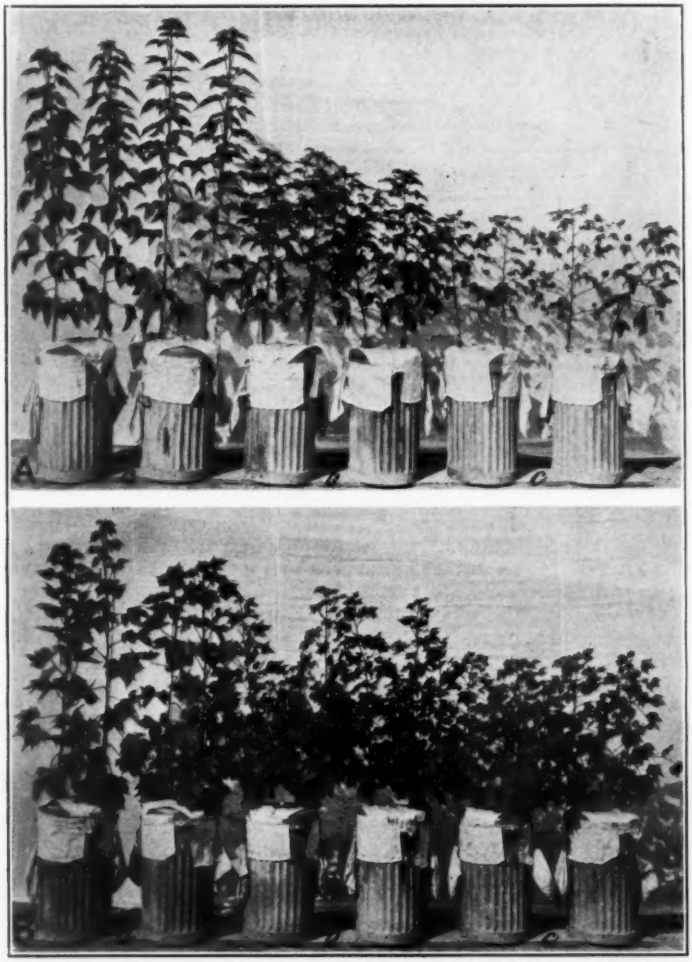


FIGURE 1.—Development of aerial parts of cotton plants: A, Pima Egyptian; B, Acala upland; A, *a*, and B, *a*, grown with neither branches nor bolls; A, *b*, and B, *b*, grown with branches but without bolls; A, *c*, and B, *c*, normal plants. Cultural treatments alike in other respects. Sacaton, Ariz., September 18, 1927. (Photographs by H. F. Loomis)

of soil. They were weighed twice each week during the growth of the plants, and water was added, usually daily, in sufficient quantity to maintain the soil moisture as nearly as possible at the moisture-

equivalent percentage, which was 16.9. The seeds were planted April 7 and the aerial parts harvested November 24. Except as has been noted, two plants were grown in each of the pots. The different treatments were replicated with 4 to 10 pots; but for each treatment, the roots in only two of the pots were separated out for examination and weighing.

After the tops had been harvested, the selected cans were placed on their sides on a rack covered with a 14-mesh wire screen. The soil was then slowly washed from the roots with a jet of water. Broken rootlets were separated from the coarse sand and gravel deposited with them on the screen by immersing the mass in a vessel of water and sieving the roots out of the agitated suspension with a small hand screen. Silt adhering to the roots after drying was removed with sieves and a fan. The aerial parts and the roots were dried to constant weight at 60° C. in an electrically heated oven. Approximately one day was required for the practically quantitative separation of the roots from the soil in each of the pots.

EXPERIMENTAL RESULTS

GROWTH OF AERIAL PARTS

Inhibiting boll development by the removal of floral buds resulted in plants of larger size and with greater leaf area. The same effect resulted also when branching was inhibited by bud removal and aerial growth limited to the main stalks and main-stalk leaves. (Table 1.) These effects are illustrated in Figure 1, from photographs taken when the plants had attained perhaps two-thirds of their ultimate growth. The stimulation of vegetative development of cotton plants, resulting from floral-bud removal, conforms with previous observations (*4*) and with results obtained by Mason (*11*). Similar effects have been reported in experiments with the tomato by Murneek (*13*) and with the grape by Chandler and Heinicke (*2*). Chandler and Heinicke's experiments resulted in moderately increased root growth, but Murneek found no effect.

The weights of the plants in pots used for analysis of distribution of dry matter between the roots and the various portions of the tops were found, after drying, to have been at variance in some cases with the means of the sets from which they had been selected. (Table 1.)

The removal of floral buds resulted in plants which elongated more rapidly and which developed a greater number of vegetative and fruiting branches and a greater leaf area than control plants. This treatment, as has been noted elsewhere (*4*), stimulates the formation of floral buds as well. Confirming the earlier results, the Pima plants in this experiment produced a mean of 240 floral buds per plant when the buds were removed, whereas the control plants produced but 49. The Acala plants produced 313 and 77 floral buds, respectively, in the two treatments.

Confining aerial growth to the main axis and its leaves resulted likewise in more vigorous plants. The rate of elongation of the main stems was greater than under either of the other treatments, and the size of the leaves of the main stems was increased. During the latter part of July the terminal buds of each of the Acala plants under this treatment ceased to elongate and develop new leaves; and the lateral buds, which if left would have produced fruiting

branches, became dominant. Many of the terminal buds were pushed to one side or produced fasciations with the branch buds. The Pima plants, without exception, were still elongating and had normal terminal buds when harvested in November, several plants by that time having attained a height of more than 2½ meters.

TABLE 1.—*Dry matter in various parts of Pima Egyptian and Acala upland cotton plants grown in pots (two plants in each pot) under indicated treatments*

PIMA EGYPTIAN

Treatment and pot No.	Pots in set	Dry matter in indicated parts of plants													Ratio	
		Tops											Roots	Total of tops and roots	Roots to tops	Tops and roots to leaves
		Vegetative parts				Reproductive parts						Mean of full set				
		Leaves	Petioles	Branches and stalks	Total	Seed cotton	Burs	Corollas	Bolls and buds shed	Total	Total					
	Number	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Normal:																
40	8	81	9	102	192	130	73	22	5	230	422	355±12.0	80	502	0.19	6.20
41		79	12	111	202	115	68	16	3	202	404		98	502	.24	6.35
Floral buds removed:																
46	4	167	19	298	484	----	----	----	[a] 19	19	503	458±14.1	237	740	.47	4.43
48		161	17	215	393	----	----	----	[b] 16	16	409		237	646	.58	4.01
All lateral buds removed:																
8	8	171	26	[c]357	554	----	----	----	----	----	554	571± 8.5	326	880	.59	5.15
9		179	29	[c]396	604	----	----	----	----	----	604		327	931	.54	5.20

ACALA UPLAND

Normal:																
52	8	93	15	93	201	164	64	14	14	256	457	454±11.0	87	544	0.19	5.85
53		93	16	86	195	185	61	13	14	273	468		70	538	.15	5.78
Floral buds removed:																
59	4	183	34	315	532	----	----	----	[a] 35	35	567	576± 8.5	255	822	.45	4.49
60		178	32	328	538	----	----	----	[b] 41	41	579		248	827	.43	4.65
All lateral buds removed: [b]																
11	10	208	45	239	[c]492	----	----	----	----	----	492	463± 6.8	330	822	.67	3.95
12		221	41	237	[c]499	----	----	----	----	----	499		286	785	.57	3.55

[a] Weight of floral buds removed.
[b] Terminal buds did not develop after Aug. 1.
[c] Stalks only; branch development has been prevented.

TABLE 2.—*Relative weights of various parts of Pima Egyptian and Acala upland cotton plants after treatments affecting fruiting and branching*

Part of plant	Relative weights of—					
	Pima			Acala		
	Normal	Floral buds removed	All lateral buds removed	Normal	Floral buds removed	All lateral buds removed[a]
Entire plants	1.00	1.38	1.80	1.00	1.52	1.49
Entire plants, less reproductive parts	1.00	2.36	3.17	1.00	2.84	2.91
Tops only [b]	1.00	1.10	1.40	1.00	1.24	1.07
Tops, less reproductive parts	1.00	2.23	2.94	1.00	2.70	2.50
Branches and stalks	1.00	2.41	3.54	1.00	3.59	2.66
Leaves	1.00	2.05	2.19	1.00	1.94	2.31
Roots	1.00	2.66	3.67	1.00	3.20	3.91

[a] Terminal buds of this Acala did not elongate after late July.
[b] The values for the full set of plants were 1.00, 1.29, and 1.61 for Pima, and 1.00, 1.27, and 1.02 for Acala.

The removal of floral buds increased the weights of the tops of Pima plants, expressed as averages of full sets, by 29 per cent and of Acala plants by 27 per cent. The increase in weight of the stalks and branches was proportionately much greater than that of the tops as a whole (Table 2), since in both the Pima and the Acala the weights of bolls of the normal plants exceeded the weights of the vegetative parts.

ROOT DEVELOPMENT

The mean ratio of roots to tops was 0.22 for the normal Pima, 0.53 for Pima without bolls, and 0.57 for Pima plants with neither bolls nor branches. In the Acala series the corresponding ratios are 0.17, 0.44, and 0.62. (Table 1.) The absence of bolls resulted not only in an increased growth of tops but also in the liberation of a relatively large proportion of material for translocation and utilization in root development.

Table 2 shows the relative weights of the various parts of the plants receiving different treatments. The increase in weight resulting from bud removal was less marked for the entire plant tops than for any of the separate parts weighed, but as has been pointed out, the bolls constituted a half or more of the weight of tops of normal plants. The two treatments in each variety, however, resulted in an increased top weight. This was not the case in experiments of Chandler and Heinicke (*2*) with grapes, in which floral-bud removal resulted in an increase in weight of stems and a moderate increase in root development, but a reduction in the weight of dry matter produced.

The removal of floral buds resulted in greater increases in the weights of branches, stalks, and roots than in the weights of leaves. Approximately proportional increases occurred in the weights of the roots and in the combined weights of the branches and stalks in each of the two varieties.

Limiting aerial growth to the main stalks and main-stalk leaves by removal of lateral buds increased the weights of Pima stalks and Pima roots to approximately equal proportions. In the Acala variety the root weights were increased relatively more than the stalks by this treatment, but the result was doubtless due to the terminal-bud abortions in August. After these buds aborted aerial growth was possible only by radial enlargement of the stalks and by increased expansion and thickening of the leaves. It is noteworthy that the suppression of branching in the Pima plants should have resulted in plants 25 per cent larger (means of full sets) than plants with branches and no bolls.

The foregoing comparisons clearly indicate that developing bolls have first call on photosynthetic products and that the bolls are markedly dominant in their growth over leaf development and stalk, branch, and root development. In the absence of developing fruit, the weight of leaves is increased less than the weight of the roots and the other woody parts of the plant. The data suggest also that branching reduces the photosynthetic output and that branch growth is partially dominant over main-stalk and root growth.

Bud removal, both floral and branch, approximately doubled the weight of leaves in both Pima and Acala. These increases in leaf weights, however, did not result in the production of proportionate increases in the weight of the entire plants. In other words, a unit

weight of leaves produced more dry matter in the normal plants than in the plants without bolls. The suggestion that increased photosynthetic efficiency results when photosynthetic products are rapidly withdrawn from leaves to fruit may explain this result in part, but in cotton a heavy setting of fruit depresses vegetative development and leaf formation. The cotton plants without fruit continued to develop new leaves (Acala without branches did not) until late in the season. The functional life of late-season leaves, however, is short and their photosynthetic contribution must be correspondingly small as compared with the contribution made by leaves developed early in the season.

Influence of Number of Plants Per Pot

The roots of two pots of Acala cotton with only one plant growing in each were removed and weighed. Table 3 shows that these single plants weighed almost as much as the combined weights of the two plants in each pot of the control set and that the weights of their roots were similarly increased. The mean ratio of roots to tops for the single-plant pots was 0.154 and for the control pots, with two plants each, 0.170.

TABLE 3.—*Dry matter in various parts of Acala upland cotton grown with two plants and with one plant per pot under normal treatment and with two plants per pot wilted before watering*

Treatment and pot No.	Pots in set	Dry matter in indicated parts of plants												Ratio	
		Tops											Entire roots	Total of tops and roots	Roots to tops	Tops and roots to leaves
		Vegetative parts				Reproductive parts						Mean of full set				
		Leaves	Petioles	Branches and stalks	Total	Seed cotton	Burs	Corollas	Bolls and buds shed	Total	Total					
	Number	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*		
Normal:																
52	8	93	15	93	201	164	64	14	14	256	457	454±11.0	87	544	0.19	5.85
53	8	93	16	86	195	185	61	13	14	273	468	454±11.0	70	538	.15	5.78
One plant in pot:																
195	6	88	17	78	183	128	54	14	16	212	395	386± 3.2	56	451	.14	5.12
196	6	84	15	64	163	140	52	12	17	221	384	386± 3.2	64	448	.17	5.33
Wilted before watering:																
149	6	62	9	51	122	97	37	11	15	160	282	217± 2.7	41	323	.15	5.21
150	6	79	10	46	135	93	40	11	17	161	296	217± 2.7	33	329	.11	4.16

Influence of Repeated Wilting

The pot-culture series included a set of Acala plants which were allowed to wilt frequently. The regular procedure with these pots was to add sufficient water after severe wilting to bring the soil moisture up to a percentage only slightly above the moisture equivalent. Under this treatment, which was started at the beginning of the flowering period in early July, severe wilting occurred as often as twice a week during August and September.

At the end of the season the frequently wilted plants weighed approximately 60 per cent as much as the normally watered control

plants. They had produced 54 per cent as much seed cotton, and their roots weighed 47 per cent as much as the roots of the control plants. The mean ratio of the root weight to the weight of tops was 0.13 for the wilted plants and 0.17 for the control plants. These ratios, which may not be significantly different, clearly indicate that frequent drying of the soil did not increase the proportion of roots. The data also serve to confirm field observations that cotton plants suffering from water shortage usually produce cotton in proportion to their size. Similar effects have appeared in pot-culture

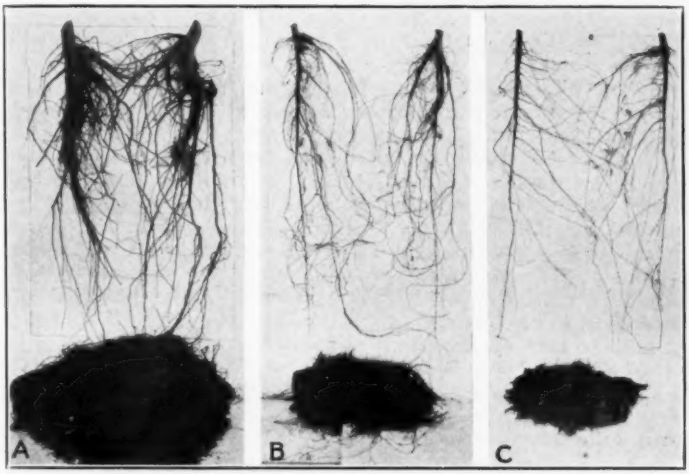


FIGURE 2.—A, Roots of Acala cotton plants grown without branches or bolls; B, roots of plants whose branching and fruiting were not interfered with; C, roots of plants that were allowed to wilt severely before each application of water. The roots are suspended within a cross-section outline of one of the pots. The masses of roots shown below were removed or broken loose from the respective pairs of plants while separating them from the soil. Sacaton, Ariz., 1927. (Photographs by H. F. Loomis)

experiments in which the plant size was reduced by salts added to the soil.

The effect of the various treatments upon root development is shown in Figure 2.

IMPORTANCE OF STIMULATED ROOT DEVELOPMENT

The relations between root development and fruitfulness as shown in the foregoing investigations may be of significance in connection with results obtained in other experiments. As a method of measuring the amount of translocation between fruiting branches, the flowers were removed throughout the season from the alternate branches of every second plant in a small planting of Acala cotton. These plants set fewer early bolls than the control plants, made a more vigorous vegetative development during the early summer, withstood to a better advantage a dry period in August, and by the end of the season had produced 9 per cent more seed cotton. The mean of the cross-section areas of the main stems, just below the cotyledon scars, of the semideflorated plants was 21 per cent greater than the mean of those of the control plants. These measurements suggest that the limited

early boll setting of the semidefloated plants had stimulated a greater root development, which contributed to the increased yield. Complete defloration during the early summer in a later experiment (*5*) resulted in increased yields for the season. Stimulated root development is considered to be but one of a number of factors, however, which contributed to the increased yields.

SUMMARY

An examination of the root systems of cotton plants grown in cans containing 110 kgm. of soil showed that root development was largely influenced by the character of the growth and the fruitfulness of the plants.

Pima Egyptian and Acala upland cotton plants were grown (1) normally, i. e., with both branches and bolls; (2) with branches but no bolls, all floral buds having been removed as they appeared; and (3) with neither branches nor bolls, the lateral buds of the main stalk having been removed as they appeared. In the Pima series the mean ratios of the weight of roots to tops were 0.22, 0.53, and 0.57, respectively, for the three treatments. In the Acala series the corresponding ratios were 0.17, 0.44, and 0.62. The data showed that the boll development of fruiting plants was dominant over both root development and vegetative growth. When plants were grown without bolls, the weight of roots increased proportionately more than the weight of tops, but the increase in weight of roots was approximately proportional to the increase in weight of stalks and branches. The weights of the roots of plants grown without bolls were approximately triple those of control plants.

Little difference was found between the proportionate weights of the roots and tops of plants grown one in a pot and those of plants grown two in a pot.

The ratio of the weight of the roots to the weight of the tops of plants allowed to wilt frequently was found to be slightly lower than the ratio of roots to tops of plants in a soil maintained as nearly as possible at an optimum moisture content.

LITERATURE CITED

(1) Balls, W. L.
1912. The cotton plant in Egypt, studies in physiology and genetics. 202 p., illus. London.

(2) Chandler, W. H., and Heinicke, A. J.
1925. Some effects of fruiting on the growth of grape vines. Amer. Soc. Hort. Sci. Proc. 22: 74–80.

(3) Collings, G. H., and Warner, J. D.
1927. Root development of cotton on Cecil sandy loam during 1926. Jour. Amer. Soc. Agron. 19: 839–842, illus.

(4) Eaton, F. M.
1927. Defruiting as an aid in cotton breeding. Jour. Heredity 18: 456–460, illus.

(5) ———
1931. Early defloration as a method of increasing cotton yields, and the relation of fruitfulness to fiber and boll characters. Jour. Agr. Research 42: 447–462, illus.

(6) ——— and Belden, G. O.
1929. Leaf temperatures of cotton and their relation to transpiration, varietal differences, and yields. U. S. Dept. Agr. Tech. Bul. 91, 40 p., illus.

(7) GARNER, W. W., and ALLARD, H. A.
1923. FURTHER STUDIES IN PHOTOPERIODISM, THE RESPONSE OF THE PLANT TO RELATIVE LENGTH OF DAY AND NIGHT. Jour. Agr. Research 23: 871–920, illus.
(8) KING, C. J.
1923. WATER-STRESS BEHAVIOR OF PIMA COTTON IN ARIZONA. U. S. Dept. Agr. Bul. 1018, 24 p., illus.
(9) MASKELL, E. J., and MASON, T. G.
1929. STUDIES ON THE TRANSPORT OF NITROGENOUS SUBSTANCES IN THE COTTON PLANT. I. PRELIMINARY OBSERVATIONS ON THE DOWNWARD TRANSPORT OF NITROGEN IN THE STEM. Ann. Bot. [London] 43: [205]–231, 615–652, illus.
(10) ——— and MASON, T. G.
1929. STUDIES ON THE TRANSPORT OF NITROGENOUS SUBSTANCES IN THE COTTON PLANT. III. THE RELATION BETWEEN LONGITUDINAL MOVEMENT AND CONCENTRATION GRADIENTS IN THE BARK. Ann. Bot. [London] 44: 1–29, illus.
(11) MASON, T. G.
1922. GROWTH AND CORRELATION IN SEA ISLAND COTTON. West Indian Bul. 19: 214–238, illus.
(12) ——— and MASKELL, E. J.
1928. STUDIES ON THE TRANSPORT OF CARBOHYDRATES IN THE COTTON PLANT. II. THE FACTORS DETERMINING THE RATE AND THE DIRECTION OF MOVEMENT OF SUGARS. Ann. Bot. [London] 42: [571]–636, illus.
(13) MURNEEK, A. E.
1925. THE EFFECTS OF FRUIT ON VEGETATIVE GROWTH IN PLANTS. Amer. Soc. Hort. Sci. Proc. (1924) 21: 274–276.
(14) WEAVER, J. E.
1926. ROOT DEVELOPMENT OF FIELD CROPS. 191 p., illus. New York.
(15) ——— and BRUNER, W. E.
1927. ROOT DEVELOPMENT OF VEGETABLE CROPS. 351 p., illus. New York.

CROWN ROT OF JUGLANS IN CALIFORNIA[1]

By CLAYTON O. SMITH, *Associate in Plant Pathology*, and JAMES T. BARRETT, *Professor of Plant Pathology and Plant Pathologist, California Agricultural Experiment Station*

INTRODUCTION

The growing of selected varieties of the English or Persian walnut, *Juglans regia* Linn., has necessitated the finding of suitable rootstocks. Often in the past the English root has not been available as a stock and has not been fully tested, except in seedling orchards. The eastern black walnut, *J. nigra* Linn., because of its slow growth in the nursery, can hardly be regarded as a commercial stock under California conditions. The California black walnuts, *J. californica* Wats. and *J. hindsii* Sarg., have been almost universally used as rootstocks for *J. regia*. A fungus disease has appeared which attacks the crowns and roots of these stocks. It is widely distributed and is assuming great economic importance. Walnut trees after reaching bearing age may die suddenly. Usually several in the same part of the grove are attacked. This paper reports and describes experiments dealing with this disease.

HISTORY, DISTRIBUTION, AND CONDITIONS FAVORING CROWN ROT

The first reference to the occurrence of crown rot, or wilt, of Juglans was made by R. E. Smith and others (*14*)[2] and concerned its appearance in southern California black walnut nursery trees. One severe case was noted as occurring on poorly drained soil where an excess of water had been used.

A root disease of the English walnut in the Brighton district of Victoria, Australia, was investigated by Cookson (*3*). The disease was found attacking the roots and crowns of English walnut trees that showed a yellowing of their foliage. Irregular black areas were found on the surface of the trunk at or just below the soil level, and a dark fluid often accumulated in cavities in the cambial zone or exuded to the surface. The infected roots were never found to be girdled, and usually only a small part of the circumference was infected. A species of Phytophthora was isolated from the roots and studied in cultures. Successful inoculations were made in the roots of 18-month-old English walnut seedlings. The fungus was provisionally determined to be *Phytophthora parasitica* Dastur.

The crown-rot disease has undoubtedly been present in the walnut nurseries of California, yet its injury has been so limited as to be of little economic importance. The loss of a few trees in a large nursery would be attributed to various other causal agencies. The disease has been more or less prevalent in the walnut groves of California for several years and is now widely distributed, having been found to some extent in most walnut-growing districts. In 1921 the disease became

[1] Received for publication Apr. 18, 1931; issued December, 1931. Paper No. 242, University of California Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, Calif.

[2] Reference is made by number (italic) to Literature Cited, p. 903.

of sufficient importance to demand special study. Investigations were started in 1923 and have continued during the last seven years.

The occurrence of the disease is usually associated with an excess of soil moisture that has continued for some time near the crown of the tree. This condition may result from improper irrigation, from flood water after heavy or long-continued rains, or from poor surface drainage. The flat areas, the lower parts of the orchard and the upper parts, where the irrigation water has to make a long run, are the places where crown rot may be expected to occur.

Some of the macroscopic characteristics of crown rot of Juglans are apparently very similar to those of the European chestnut ink disease, caused by *Phytophthora cambivora* (Petri) Buis., as described in the writings of Dufrénoy (*4*) and Petri (*11*). The diseased tissues of the walnut and the chestnut each give an exudate that becomes black upon exposure. Mature trees of each die after their crowns and roots are attacked and killed by their distinctive species of Phytophthora.

FIGURE 1.—Two English walnut trees on *Juglans californica* root, both affected with crown rot and showing the characteristic thin foliage. A, Tree with crown affected on side corresponding with thin foliage. (See Fig. 2, A.) B, Tree on which were made the inoculations shown in Fig. 9

SYMPTOMS OF CROWN ROT

Trees affected by crown rot are not easily distinguished from those not affected, and an inspection of the crown of the tree is usually necessary for its positive determination.

The first indication of infection is often a somewhat stunted appearance of the tree, accompanied with sparse foliage (fig. 1) of a yellowish tint. The yield of nuts may be exceptionally heavy during the initial stages of the disease. In more advanced stages of the disease the tree appears drought stricken during the early growing season, and in midsummer the leaves turn yellow and drop off leaving the crop of nuts exposed to shrivel. Usually the nuts drop that season but may remain until the following spring, when the tree may leaf out, but the tree usually dies before the second season is over. On examination the crown is found to be girdled.

The infected tissue is firm, and when cut has a dark, moist appearance. The disease does not in any way resemble a soft rot.

The blackened diseased tissue is often entirely restricted to the tissue of the black walnut rootstock where it may extend downward on the roots for some distance (1 to 3 feet). While the infection may be limited above by the graft union, it frequently extends into the English walnut tissue (figs. 2 and 3), progressing slowly at first, but more rapidly as the crown tissues are severely infected and practically girdled. The infection may extend upward in the trunk to a height of 4 feet (fig. 4), and if the tree does not die too quickly, it may extend into large branches. The infected tissue has been traced for a distance of 8 feet, from the crown through the trunk into the large branches.

The spread of the disease into healthy tissue often takes place by the development of a narrow streak of infected, dark-colored tissue, extending some distance (sometimes several feet) from the initial lesion. The advance of these lesions is at first superficial, usually only the outermost layer of living bark being invaded. Later the entire bark becomes infected. While the disease, in many cases, may seem to develop suddenly, careful observations of many infected trees show that in general its progress is comparatively slow. Field observations indicate that the disease develops most rapidly under moist, cool conditions, and that it becomes inactive and makes little progress during the warm, dry part of the season. The disease progresses slowly from year to year until some unusually favorable condition permits a rapid spread of the pathogene and finally a girdling of the tree. The gradual decrease in vigor of the infected tree may also favor a rapid development of the disease.

The disease for the most part affects the bark only, but can stain the wood. According to Smith and Smith (*16*), the disease sometimes actually invades the wood, where at scattered points it can live through unfavorable conditions. There exudes from the diseased tissue a liquid which, upon exposure to the air, forms a conspicuous black paintlike coating extending down from the lesion for several feet. (Fig. 5, A.) This is called "black sap" and is easily recognized on the white trunk of the English walnut, but because of absorption by the soil it is more difficult to detect on the underground bark of the black walnut rootstocks.

The disease closely resembles another walnut disease, described by Fawcett (*6*) as Melaxuma, in that both have a black-sap exudate. Crown rot is distinguished from Melaxuma principally by the location of its lesion, which is found on the English walnut trunk extending upward from the black walnut crown. Melaxuma, being a disease of the English walnut rather than of the black walnut, is found attacking the branches as well as the trunk, especially the crotches of the large limbs.

MICROSCOPIC STUDY OF THE DISEASED TISSUE

Radial microtome sections were made of the bark taken from the margin of an artificial lesion (fig. 6, A) on the trunk of a southern California black walnut 5 inches in diameter. The sections were cleared in a lacto-phenol solution and stained with 0.1 per cent cotton blue in the same solution. The diseased, brownish-colored walnut tissue, as well as hyphae in it, often did not take the cotton-blue dye well, and the cell walls and hyphae remained a brownish color. In the healthier parts of the same sections the hyphae were readily

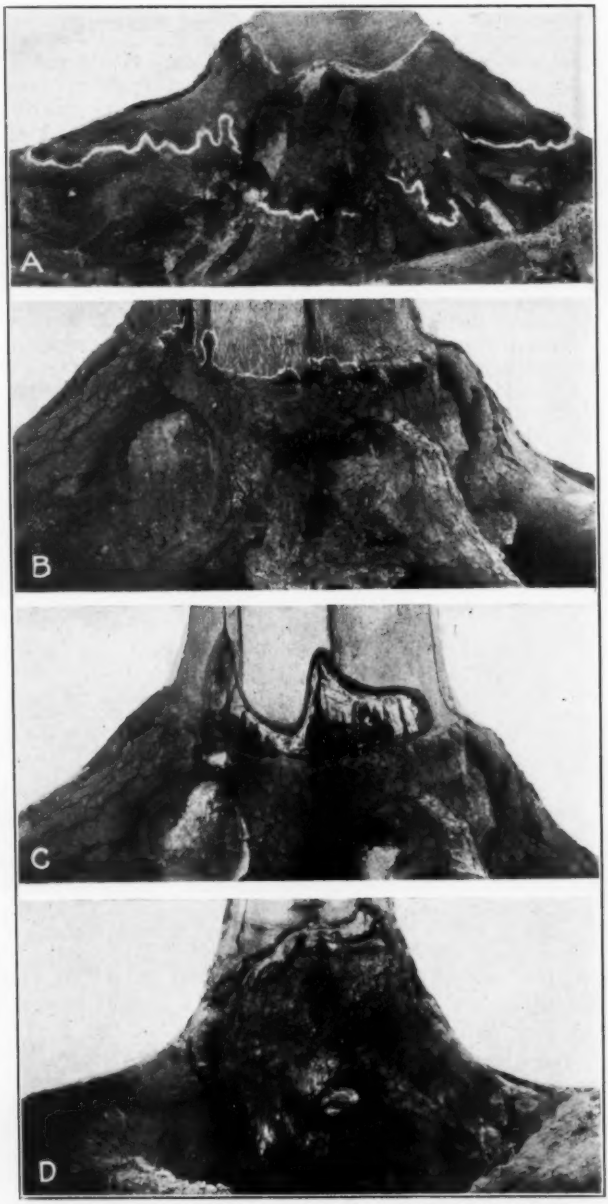


FIGURE 2.—Natural infection on crown of southern California black walnut. A, Crown partly girdled. (See Fig. 1, A, for condition of foliage; outline indicates margin of infected area.) B, Crown partly infected as outlined; margin extending somewhat into English walnut bark. C, Same tree as shown in Fig. 2, B, after cutting away the bark to show greater extent of invasion into English walnut bark than Fig. 2, B, would indicate. D, Large lesions extending about half the distance around crown and some distance into the English trunk

stained. The nonseptated hyphae were intercellular (fig. 6, B–D) and occasionally intracellular (fig. 6, E) and were readily recognized by their blue or brownish color. They were found in the brownish

FIGURE 3.—A, Trunk lesion in English walnut from which Phytophthora was isolated. Photographed in May. B, Same tree as in A; note *different* advances of disease as indicated by lines, from May to October, when photographed. C, Tree in which infection started in slit of bark

diseased tissue and for a short distance (5 mm.) in what appeared to be healthy green tissue. Stained cross and radial sections of the wood covered by this bark did not show hyphae. A cross section of the

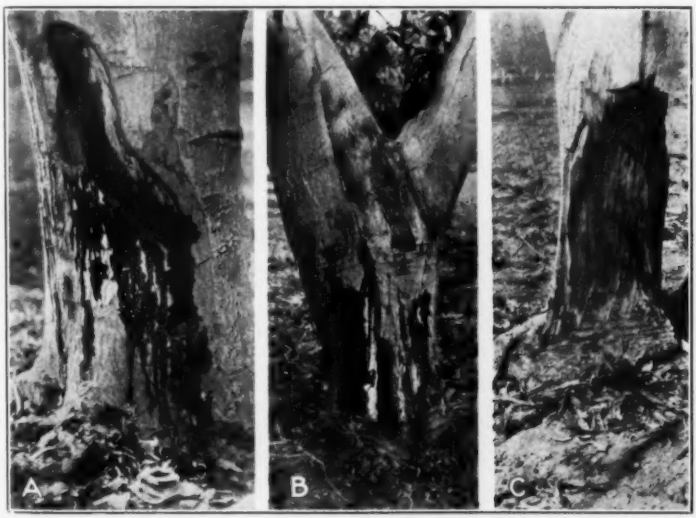


FIGURE 4.—Trunk lesions on English walnut; Phytophthora isolated from A and B. Bark partly cut away in A to show dark stain of wood. C shows a lesion 3 feet long after most of the diseased bark had been removed

wood taken from an artificial lesion of an English walnut also failed to show hyphae, but they were found in radial sections of the bark over this wood. In both cases the hyphae were found between the parenchyma cells of the cortex, were abundant in the secondary

phloem, and occasionally present in the phloem rays (medullary rays). Radial sections from a natural lesion on a large (10 inches in diameter)

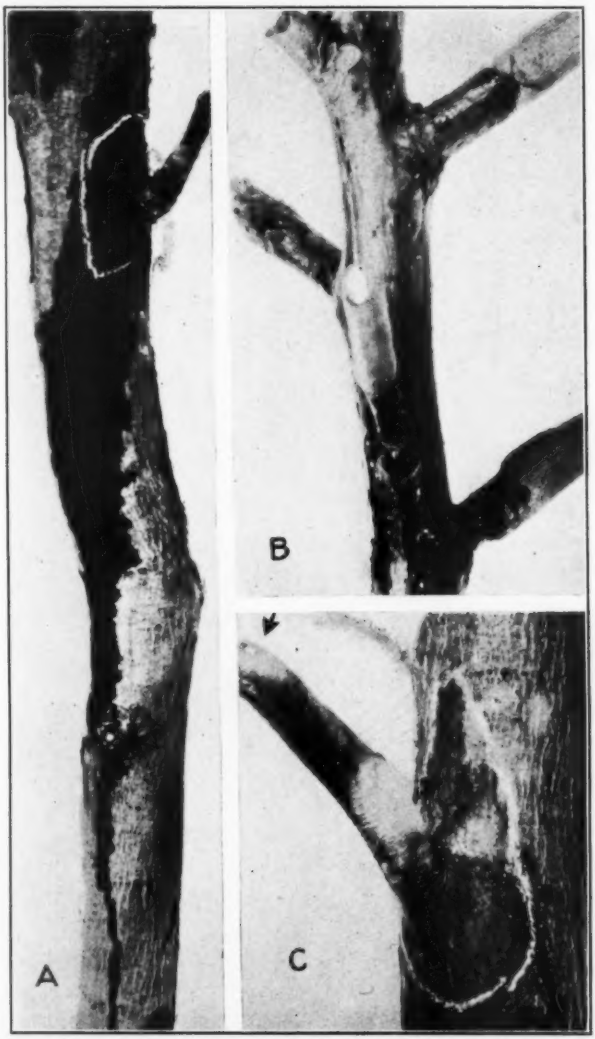


FIGURE 5.—Artificial inoculations on English walnut seedlings. A, Inoculation by Phytophthora in small side branch at right, forming a lesion as outlined, 5 inches in diameter; also black sap. B, Inoculation by Phytophthora on 2-year old English walnut seedling; photographed after 60 days; shoot and side branches are girdled. C, Inoculation by Pythiacystis type on side branch at point indicated by arrow spreading to trunk

branch of an English walnut showed intercellular mycelium in phloem rays and parenchyma. (Fig. 6, F–H.) From this lesion Phytophthora was isolated. These proofs of the mycelium in the tissue are

in close agreement with that of Cookson (*3*) who further observed the hyphae as occurring occasionally in the wood vessels and in the wood fibers of an English walnut root artificially infected with *Phytophthora parasitica*.

ISOLATION OF ORGANISM

Successful isolations of the crown-rot organisms have been reported by Barrett and Smith (*1*). They list two fungi which have a causal relation to the disease, a species of Phytophthora of the Pythiacystis type and a species of Phytophthora now known to be *Phytophthora cactorum* (Leb. and Cohn) Schröt. A fungus similar to the latter, if not identical, was isolated by Smith and Smith (*16*) from *Juglans hindsii* and was referred to *P. cactorum*.

Since these papers were published and during the present study, four different isolations of the walnut crown-rot fungus (the cultures more frequently used in the artificial inoculations) were sent for study to S. F. Ashby, Imperial Mycological Institute, Kew, England. His conclusions were that all four cultures belonged to the same species which he believed to be *Phytophthora cactorum* (Leb. and Cohn) Schröt. Ashby's studies confirmed the provisional determinations made by Smith and Smith (*16*) and Barrett.[3]

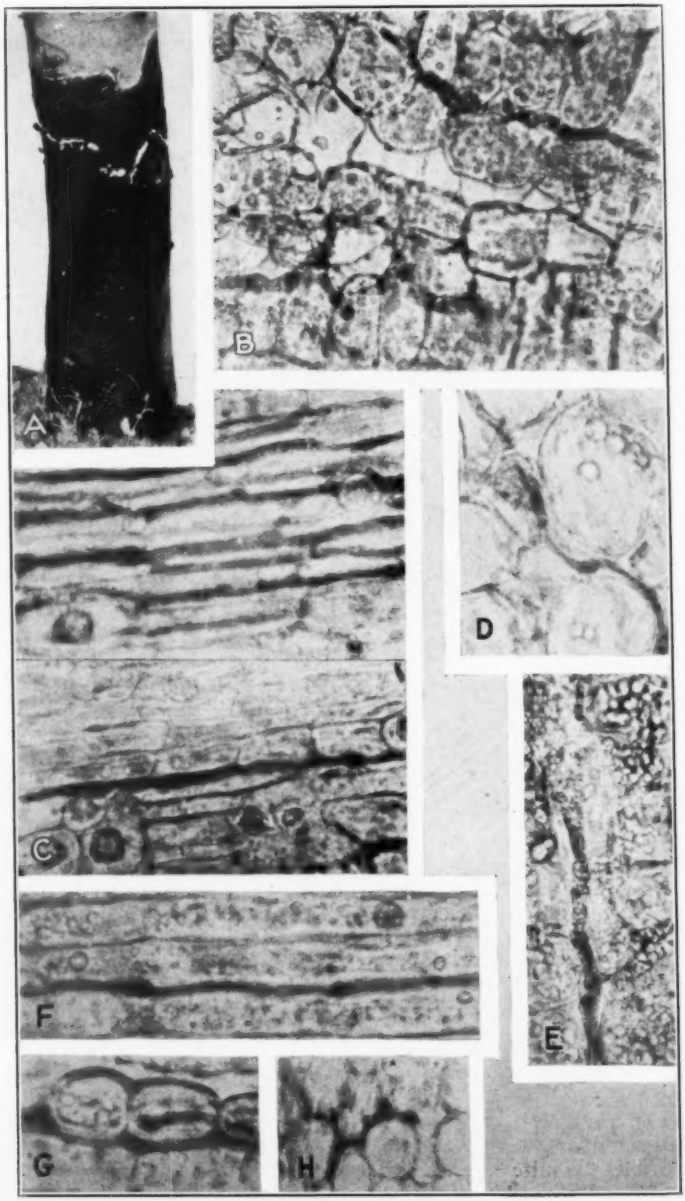
The genus Pythiacystis was formed to include types of Phycomycetes intermediate between Pythium and Phytophthora (*13*, *15*); and *Phytophthora* (*Pythiacystis*) *citrophthora* (Smith and Smith) Leonian was the type species. A more complete knowledge of this and allied species, especially that of *P. cactorum*, seems to indicate that no consistent differences exist between the two genera except an absence of oospores in Pythiacystis. Phytopthora is now generally used to include the one described species of Pythiacystis and is so recognized by Smith and Smith (*16*, *p. 398*). This later term is retained in this paper with its original meaning—an absence of oospores.

The crown-rot organism is isolated with some difficulty, especially from the California black walnut roots. In this study more than 325 attempts were made to isolate a pathogene from the crown-rot tissues of *Juglans californica*. The results were 165 sterile cultures, 109 cultures of bacteria, and 50 of miscellaneous fungi, and 1 isolation of a Phytophthora of the Pythiacystis type.

Reisolations from artificial inoculations on black walnut tissue are made with great difficulty. Some successful recoveries of the organism have been made, but only after several cultures. This difficulty is now known to be due to a toxic exudate from the tissue of black walnut when cut. (Table 1.) Water blanks and media are quickly stained a dark color when thin pieces of bark are added. No way has been found to overcome this difficulty, nor has it been determined what this toxic substance is, but it made impossible the successful following of Koch's rule of proof.

The causal organism is more successfully isolated from the infected English walnut tissue, but even in this species it has not been isolated from all parts of the diseased area. In this study 227 attempted isolations were made from English walnut trunks affected with crown rot. These resulted in 74 sterile cultures, 52 showing bacteria, 70 showing fungi of the well-known saprophytic types, and 31 cultures

[3] The report of the University of California Agricultural Experiment Station for 1927–28 (*10*, *p. 40*) gives the causal organis for crown rot as Phytophthora, probably *P. cactorum*.



(For explanatory legend see opposite page)

of Phytophthora. Other isolations of the Pythiacystis type (not included in the above) were obtained from watery black-sap pockets on the trunks of English walnut trees. When these cultures were inoculated into Juglans, they were pathogenic.

Phytophthora cactorum was isolated from the English walnut trunk (fig. 4) infected from the diseased black walnut crown, and is regarded as the causal organism of crown rot. Isolations are successfully made from the active margin of the lesion. In the older diseased areas, the organism has become inactive or is entirely dead. The activity of the disease near the margin is further shown from some observations on pieces of English walnut bark that were affected with crown rot and had been wrapped in paraffin paper for a few days. In a place near the margin of the lesion, hyphae of what appeared to be Phytophthora were found growing from the bark. Transfers to culture media proved the correctness of this observation. In similar specimens of English bark a Phytophthora was found developing as a frostlike growth that showed oogonia in several places on the cut surface and outer bark layers.

TOXICITY OF WALNUT BARK EXUDATE

An experiment to test the effect of small amounts of walnut tissue on mycelial growth of the walnut Phytophthora was performed as follows: Fifteen cubic centimeters of glucose-potato agar and 1 gm. of walnut bark were placed in culture tubes and boiled. The kinds of bark used in these tubes were a white, hard, healthy English walnut bark, diseased English walnut bark, healthy southern black walnut bark from crown, and diseased southern black walnut bark from crown. A tube containing no bark was used as a control. After boiling, the agar was poured into sterile Petri dishes and allowed to harden. Each plate was then inoculated with a culture of Phytophthora of the cactorum type and a culture of Phytophthora of the Pythiacystis type. Small pieces of agar containing the mycelium were placed on opposite sides of the Petri dish about equidistant from the middle.

The results are given in Table 1. They show that the healthy bark of the southern black walnut is more toxic than that of the English walnut and less so than diseased southern black walnut bark. The diseased English walnut bark is slightly less toxic than the healthy bark.

This preliminary experiment was repeated, using diseased bark from the artificial inoculations with Phytophthora on English walnut and southern California black walnut, and the results were substantially as given in Table 1.

EXPLANATORY LEGEND FOR FIGURE 6

FIGURE 6.—Artificial inoculations and hyphae in tissue. A, *Juglans californica* showing lesion from an artificial inoculation on crown underground. The 5-inch trunk is girdled and the lesion has advanced 10 inches above ground. The upper margin is indicated by the lower white line with black sap above. Ten reisolations of the causal organism were made from this lesion. B–E, Radial sections from upper margin of the lesion in A. B and D show intercellular hyphae in parenchyma, with collapsed infected cells also to be seen in D; C, intercellular hyphae in phloem rays; E, intercellular and intracellular hyphae in parenchyma. F–H, Radial sections from tissue at the upper margin of a natural lesion (8 feet from ground) on a large English walnut limb 10 inches in diameter. Conditions were much like those shown in Fig. 4. F shows intercellular hyphae in phloem ray; G, intracellular hyphae in cell; H, intercellular hyphae in parenchyma. (Microscopic photographs by L. J. Klotz)

TABLE 1.—*Toxic effect of walnut bark on the mycelium of Pythiacystis and Phytophthora from Juglans*

[Tests were made by placing a small piece of agar containing mycelium of Pythiacystis near one edge and another piece containing mycelium of Phytophthora near the opposite edge of the medium in a Petri dish]

Bark [a] and condition	Growth in 13 days after inoculation with—		Growth in 20 days after inoculation with—	
	Pythiacystis	Phytophthora	Pythiacystis	Phytophthora
	Cm.	*Cm.*	*Cm.*	*Cm.*
English walnut, healthy	2	[b]	2.25	1
English walnut, diseased	4	4	[c] 6.25	6
Southern black walnut, healthy	1.5	[d]	1.7	[d]
Southern black walnut, diseased	[e]	[e]	.1	.1
Control	[f]	[f]		

[a] 1 gm. of bark boiled with 15 c. c. of glucose-potato agar and cooled in Petri dishes.
[b] No growth.
[c] Colonies touching and hyphae intermingled slightly. At this point oospores are abundant 5 mm. back in the *Phytophthora* colony as well as near walls of Petri dish.
[d] Slight growth.
[e] Trace.
[f] Combined growth covered Petri plate.

Dilutions of this agar in which 1 gm. of walnut bark had been boiled were made so that the amount of bark extract would represent that which would be obtained from the following portions of a gram of walnut bark: One-thirtieth, one-fifteenth, two-fifteenths, one-third, seven-fifteenths, one-half, nine-fifteenths, eleven-fifteenths, and thirteen-fifteenths. Tests were made (as in former experiments) by inoculating the hardened agar in Petri dishes with mycelium from cultures of the *cactorum* and Pythiacystis types of Phytophthora.

In the plates containing one-thirtieth and two-fifteenths of a gram of walnut bark the growth was at first retarded, but at the end of 10 days it was as great as that in the control plates. Some reduction in amount of growth (9 to 35 mm. as compared with 50 to 60 mm. in control plates) was noted in the plates containing one-third of a gram. In the remaining dilutions (seven-fifteenths, one-half, nine-fifteenths, eleven-fifteenths, and thirteen-fifteenths of a gram), a smaller but more uniform amount of inoculum was used. A 3-mm. disk of agar was cut from the mycelial growth of the fungus on an agar plate and was transferred to the plates to be tested. At the end of 10 days the growth in the control plates had coalesced and measured 45 to 70 mm. in diameter.

In the plates containing the extract from seven-fifteenths of a gram of walnut bark, the growth with the healthy English bark was 7 mm., and with the healthy southern black walnut 25 mm. In one plate containing one-half of a gram of healthy southern California black walnut bark there was a 13-mm. growth. In all the remainder of the series (nine-fifteenths, eleven-fifteenths, thirteen-fifteenths, and 1 gm. each of healthy and diseased English walnut bark and healthy and diseased southern California black walnut bark) there was no measurable growth. A slight growth from the inoculation disk was observed where they were not immersed in the agar. In the Petri plates containing nine-fifteenths to 1 gm. of walnut bark, where growth of Phytophthora was inhibited, contaminations by air-borne spores of Alternaria, Aspergillus, and Penicillium were able to make vigorous growth.

ARTIFICIAL INOCULATIONS

All the successful inoculations on Juglans were made in wounds. In the first test they consisted of cuts by knife or scalpel into the bark, which was loosened sufficiently so that all the mycelium from one culture tube could be inserted if desired. The aim was always to use an abundance of inoculum, usually all the growth from the surface of a single culture tube. The method was later refined by using an opening of a constant size made by a one-fourth-inch cork borer. The opening was filled with mycelium: the bark plug was sometimes reinserted and sometimes omitted. The openings were covered with paraffin paper fastened with waterproof glue. Adhesive tape for covering the wounds was later substituted with satisfactory results. (Fig. 7.)

The inoculations were made chiefly with isolations of Phytophthora from *Juglans regia*, but some were made with cultures from *J. californica* and from *J. hindsii*. The cultures were mostly of the *Phytophthora cactorum* type with some local isolation of Phytophthora of the Pythiacystis type. The reaction of Juglans with these two types showed practically no difference in infection on the species tested. Cultures of *P. nicotianae* from tobacco and cultures of *P. cactorum* from other hosts were also tested. All the cultures of these species gave typical lesions on *J. californica*, as recorded in Table 2. The inoculation with both types of the Phytophthora from Juglans indicate that all the species of Juglans tested are more or less susceptible when artificially inoculated. The following is a tentative arrangement of the species in descending order of susceptibility: *J. californica*, *J. regia*, *J. hindsii*, and Paradox hybrid (*J. californica* × *regia*). *J. sieboldiana* and *J. nigra* can readily be infected but have not been sufficiently tested to judge of their relative susceptibility.

TABLE 2.—*Results of inoculations on Juglans californica with various organisms in series of five punctures*

Organism	Period elapsed [a]	Source		Radius of lesions in millimeters	
		Isolation	Place or person	First to fifth inoculations in order	Average
	Days				
Phytophthora cactorum	40	Pyrus communis	Butler, L. R.	[b] 80	100
	90	do	do	40–55–20–30–0	36
	40	Rheum rhaponticum	Beach, W. S.	50–40–50–15–0	39
	90	do	do	20–65–80–0–60	56
	40	Fragaria (fruit)	Rose, D. H.	0–0–100–70–50	73
	40	do	do	0–0–100–40–35	58
Phytophthora sp	40	Juglans hindsii	California	60–45–60–0–0	55
	40	J. regia	do	60–40–80–65–80	65
	90	do	do	10–15–0–15–15	14
	90	Nicotiana	Reddick, D.	25–20–0–0–10	18
Nicotianae	40	do	Florida	0–0–0–0–10	10
P. nicotianae	90	do	Reddick, D.	0–0–10–10–10	10
P. nicotianae, var. tabificae.	40	do	do	30–15–10–40–20	23
Pythiacystis citrophthora	40	Citrus	California	0–90–110–80–65	86
Pythiacystis type	40	Capsicum	do	40–40–10–70–65	45
	90	Persea (fruit)	do	120–120–130–0–0	123
	90	Citrullus (vulgaris fruit)	do	20–25–0–80–110	59
	40	do	do	20–15–10–0–0	15
	90	Rheum rhaponticum	do	[c]	95
	90	do	do	90–55–60–10–180	73
	40	J. californica	do	50–60–70–0–0	60
	40	J. regia	do	75–75–30–60–80	64
	40	do	do	80–90–120–100–50	88

[a] The inoculations extending over the 40-day period were made June 18, 1930; those of the 90-day period were made July 1, 1930.

[b] Four lesions have coalesced and measure 820 mm. One inoculation negative.

[c] Girdled, 950 mm.

FIGURE 7.—A, Inoculations with *Phytophthora cactorum* type on small limbs of an English walnut seedling; the lesions are 70 to 150 mm. long. X indicates where bark was removed with cork borer. B, Inoculation on small limbs of English walnut with the Pythiacystis type of Phytophthora. Both A and B photographed after 11 months

Experiments in placing mycelium from pure cultures on the uninjured surface of bark of southern California black and English walnuts gave negative results. Two methods were tried. In the first the mycelium was placed on the surface and covered with moist cotton which was tied in place. This was covered with sphagnum moss, and the whole wrapped with paraffin paper. A funnel was placed in the sphagnum for adding water when necessary. In the second method, the mycelium was placed in a rubber washer made from a cross section of rubber tubing. The rubber washer was fastened on adhesive tape, a disk of paraffin paper first being placed inside the washer, and then the mycelium was made to fill this infection chamber. The whole was then fastened by adhesive tape on the trunk of small seedlings (2 to 3 inches in diameter) of English walnuts and southern California black walnuts. Negative results were obtained in 15 trials on each of the walnuts.

The first of the methods described was modified by making a 1-mm. puncture through the bark of the trunk of English walnut seedlings and southern California black walnut seedlings that were 2 to 3 inches in diameter. After 3 weeks, 6 inoculations on the southern California black walnut gave 6 lesions one-fourth to one-half inch in diameter. Six inoculations on English walnuts gave 3 negative and 3 positive lesions, 1 inch in diameter.

Some other tests on small tree trunks were made by scraping the surface from the bark of English walnut and southern California black walnut. A circle one-third of an inch in diameter was thus scraped, the mycelium was held in place with wet cotton, and the whole protected with moist sphagnum and paraffin paper. After 30 days the following results were observed: 6 inoculations of southern California black walnut showed 1 failure and 5 positive lesions one-fourth inch to 3 inches in size. The results on English walnut from 15 inoculations were 4 negative and 11 positive lesions one-half inch to 1 inch in size. They extended through the bark and stained the wood.

The number of inoculations made during 1928–29 are given in Table 3 under 1929. Phytophthora was inoculated in wounds cut through the bark of Juglans by a one-fourth inch cork borer. The inoculations were covered with adhesive tape. They were made in trees 4 to 6 years old, in series of five on each tree, in tissue 2 to 4 inches in diameter, and at frequent intervals (two to four weeks) from November 1 to June 15. They at first showed much activity and formed lesions with a radius varying from 5 to 125 mm., but at the close of the growing season the lesions were for the most part inactive, and many had healed. Under the conditions of the experiment there was little consistent difference in size or number of lesions formed at different periods of the year. March and April were somewhat more favorable for infection. The general results and the condition of lesions as they were observed at the end of the experiment may be summarized as follows:

The inoculations in the northern California black walnut seedlings showed that 130, or practically the entire number, had healed. No girdling or large lesions were found.

On southern California black walnut seedlings 314 inoculations were made. Many had become checked, and some had healed. Fourteen southern California black walnut seedlings with five inoculations each were girdled and had died above the lesions.

On English walnut seedlings, 144 inoculations caused 132 lesions 1 to 4 inches in diameter. The lesions were larger (4 to 8 inches long) on the young shoots that measured 1 to 1.5 inches in diameter. In 23 series of 5 inoculations each there was no girdling, while in 6 series of 5 inoculations there was girdling of the smaller shoots.

SUSCEPTIBILITY OF JUGLANS CALIFORNICA AS SHOWN BY INOCULATIONS

The preliminary inoculations in this study were made on a large limb of a 20-year-old southern black walnut tree (fig. 8) using a culture of *Phytophthora* (*Pythiacystis*) *citrophthora* isolated from Valencia orange fruit. In 60 days the 2 lesions measured 1.5 to 3 by 6 to 12 inches with much black sap on the surface. The first positive inocu-

FIGURE 8.—Two different views of a 20-year-old southern California black walnut on which the preliminary inoculations were made. The large lesion resulted from the coalescence of two lesions and is 40 inches long and two-thirds the distance around the 5-inch limb. The inoculation was by Pythiacystis type and this organism was reisolated from the lesion. A, Smaller lesion on another limb made also by Pythiacystis; B, another lesion (inoculated with both Pythiacystis and Phytophthora) near where limbs branch from trunk; this has invaded and girdled the left central branch

lations with cultures isolated from Juglans were obtained on this same southern black walnut on March 3. In 90 days the lesion was two-thirds of the distance around a 5-inch limb and 24 inches long. The surface of infected, as well as of the healthy bark, was coated with the black-sap exudate. These preliminary results were duplicated many times.

Some inoculation tests of special interest were made on southern California black walnuts growing in their native habitat. These trees are of unknown age and about 10 to 15 feet in height.

The sizes of the stems inoculated varied from 3 to 12 or more inches in diameter. After four months the largest lesions on the trunks were about 9 to 22 inches long. These lesions continued to increase from March to May, when they were 18 to 30 inches long, and the following

October, a year after they were made, they were over 6 feet long. Another inoculation made on the trunk from the same culture at the same time had within one year produced a lesion 8 feet long and had killed the trunk, which was 8 inches in diameter.

A few inoculations were made below ground on the crown of two southern California black walnut trunks which were grafted to English walnuts. These were made on March 5, and after eight months had developed into a diseased zone 3 to 6 inches broad, extending two-thirds the distance around the tree and up to the bud union, but not into the English walnut bark. Small nursery trees of the southern California black walnut when inoculated have frequently been killed by girdling. (Fig. 6, A.) Sometimes the action of the fungus in the aerial inoculations is not so virulent and small lesions 2 to 4 inches in size may develop, which during hot weather cease to increase in size.

RESISTANCE SHOWN BY THE NORTHERN BLACK WALNUT

The first successful inoculations with *Phytophthora* sp. in *Juglans hindsii* were described by Smith and Smith (*16*). The present writers' inoculation tests on this host agree well with theirs, and the lesions are never so large as those just described as produced in *J. californica*. In some of the earlier experiments the percentage of positive lesions artificially produced in the northern California black walnut were about half those produced in the southern California black walnut. (Table 3.) In the work of 1929 there was little difference in the percentage of lesions produced in the two hosts, but the difference in size and the more rapid development of the disease in the southern California black walnut seemed to persist. On an 8-year-old northern California black walnut tree growing in the orchard at the citrus experiment station 28 inoculations gave 23 negative results and 5 lesions one-fourth inch to 2 inches in length. These never increased in size and are in marked contrast to the large lesions, 6 to 8 feet long, reported on the old southern California black walnut trees at Whittier and in Brea Canyon.

TABLE 3.—*Results of artificial inoculations on Juglans by Phytophthora and Phytophthora (Pythiacystis type)*

[Number of negative and positive results given]

Year	Southern California black walnut		Northern California black walnut		English walnut		Paradox hybrid	
	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive
1924	0	7	22	15	2	17	4	9
1925	10	42	56	32	5	23	33	9
1926	10	3	40	5	6	5	----	----
1927	0	3	15	2	4	8	----	----
1928	13	37	16	47	3	32	----	----
1929	29	183	31	94	13	132	----	----
Total	62	275	180	195	33	217	37	18
Total positive, per cent	82		52		87		32.7	
Total positive in 1929, per cent	82		75		91		----	

In some inoculations, on the crown of 2-year-old northern California black walnut trees at the time of replanting, the following results were observed by Barrett: Single inoculations were made on each of 16 trees. Three different isolations of Phytophthora from Juglans were used in the experiment. Eight months after inoculation each of the three organisms had produced lesions. The results showed that 7 trees had been girdled on the taproot at the point of inoculation, that 2 of these had died before tree growth in the spring had begun, and that 2 other trees had developed typical lesions. Seven other trees showed negative results. Five of the girdled trees produced strong lateral roots above the lesion. At the end of 3 years the lesions on trunks of 2 trees had increased in size, and 2 of the girdled trees showed other lesions on the lateral roots. The results

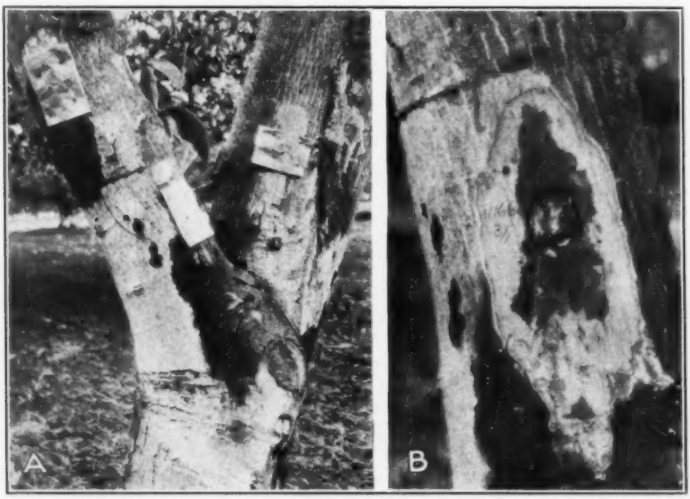


FIGURE 9.—A, Results after 60 days from two artificial inoculations (3 by 7 to 8 inches) by Phytophthora on large limbs of an English walnut tree that was suffering a decline from this disease at the crown. (See Fig. 1, B, which shows this tree with thin foliage.) B, An enlargement of the lower lesion of A

on 9 trees were then regarded as negative. These trees were still alive five years after inoculation, when most of them were removed.

INOCULATIONS ON JUGLANS REGIA

All field observations indicate that *Juglans regia* is free from crown rot when growing on its own roots. The crown-rot disease, however, spreads to the English walnut bark when the crown is severely diseased.

Successful inoculations (figs. 5, 7, and 9) were readily made on the trunk and limbs of the English walnut. In 90 days, lesions 3 to 8 inches in length developed, which were similar in size to those made on the northern California black walnut. The active lesions, resulting from aerial inoculations, may soon become inactive and probably die out because of the hot weather. Attempts to produce lesions in the English walnut crown by inoculation have usually been unsuccessful,

but more evidence is necessary before it is proved that this part of the tree has any particular resistance.[4] Inoculations in the thick bark of the trunk are slow in developing a lesion and give many failures, but the small branches are frequently girdled when inoculated. (Fig. 5, B.)

The largest lesion on *J. regia* in the 1930 inoculation tests was on an 8-year-old Placentia tree. The inoculation was made with Phytophthora at the margin of an old slit in the bark. (See fig. 3, C, for illustration of this method of treatment.) The wound had been made for some time, a ½-inch strip of bark having been removed. The inoculation was made April 29, and after three months had formed a lesion 3 inches wide and 12 inches long with the usual black-sap exudate. At the present time it is difficult to reconcile these positive results from artificial inoculation with the freedom of the English walnut seedlings from crown rot as shown by field observations.

INOCULATIONS OF OTHER SPECIES OF JUGLANS

The Paradox (*Juglans californica* × *J. regia*) has been tested in a limited way and shows strong resistance to any extensive development of the disease in the bark. In 55 inoculations, 18 positive lesions developed, but only a very small amount of tissue was involved (one-eighth to three-fourths of an inch), and no lesion of a large size has ever developed.

Juglans sieboldiana, the Japanese walnut, has been inoculated only a few times but gave some positive lesions, the largest being 2 by 16 inches.

Seedlings of *Juglans nigra* from a large California tree grown from a Texas nut have been tested. These trees are very variable, and many of them are hybrids. The results for 1929 showed these trees to be susceptible, as 71 lesions (one-fourth to 3 inches in size) resulted from 86 inoculations. In one case a 4-inch trunk was girdled. Too much importance should not be attached to these results because of the difficulty of knowing the exact species tested. Seedlings of *J. nigra*, grown from seed from an eastern source, have been susceptible when inoculated, as have *J. major*, Heller, *J. pyriformis* Liebm. (a Mexican species), *J. insularis* Grisebach (a Cuban species), and *J. mandshurica* Maxim. (Formosa strain).

INOCULATIONS ON OTHER HOSTS

Investigators have shown that trees of different species are frequently infected with Phytophthora or Pythiacystis type of Phytopthora. The more important ones are citrus infected with *Phytopthora* (*Pythiacystis*) *citrophthora* (*8*, *5*, *13*), avocado (*7*), deciduous nursery stock, peach, pear, almond, and apricot (*12*, *16*).

Artificial inoculations with the two forms of Phytophthora (the *cactorum* and Pythiacystis types) have shown the following plants susceptible when artificially inoculated: Almond, peach, pear, sweet cherry, French prune, *Prunus mume*, *Populus* sp., *Opuntia* sp. (cactus), *Castanea* sp., *Eucalyptus* sp. with small lesion, and lemon fruits. On

[4] Since this paper was submitted for publication the results of the 1931 inoculations have become available. These inoculations, made on the crowns of seedlings 3 to 5 inches in diameter, may be summarized as follows: *Juglans regia*—19 uninfected, 2 slightly infected, and 1 girdled on trunk above crown. *J. californica*—8 negative, 4 slightly infected, and 10 girdled, with lesions extending 1 foot above soil.

apple, quince, willow, fig, and *Carissa grandiflora* (Natal plum) the inoculations gave negative results.

CONTROL METHODS

Fawcett (*8*) has shown that tree surgery methods are successful in the control of *Pythiacystis citrophthora* (lemon gummosis). The same general methods of cutting out and disinfecting have been used in California by Braucher (*2*) for the control of crown-rot lesions of walnuts. He has also shown that simply exposing the crown by the removal of the dirt will often check the disease. This last method is much the same as that recommended by Gioda (*9*) and Petri (*11*) in the treatment of the ink disease of the chestnut in Europe.

The authors' experiments in crown-rot control were made on selected trees grafted on *Juglans californica* roots. The crowns of these black walnuts measured 1.5 to 3 feet in diameter and were diseased from one-eighth to one-half of their circumference. The treatment consisted in cutting out all the diseased tissue in some of the trees, while in others the diseased area was isolated by cutting out a groove into the healthy tissue just outside the margin of the lesion. The wounds were disinfected and allowed to dry out. The results indicate that the disease was checked. When these methods are used on small lesions they are successful. However, the size of the walnut tree and the distance to which the infection extends on the taproot are limiting factors in the practical application of these methods. Observation has shown that when carefully treated, the tissue heals over, though very slowly, and new infection does not often occur. It should be borne in mind that such treatment of large trees is difficult, slow, and costly. Complete tree surgery, that is, cutting out all the diseased tissue, should be resorted to only in those cases that do not respond to drying out accomplished by the removal of earth from the crown roots.

Experimental inoculations and field observations by the authors indicate that the English walnut root is resistant to crown rot under the usual orchard conditions. The selection of a suitable English walnut root in new plantings will do much to obviate future crown-rot trouble.

It has already been shown that much of the trouble may be avoided by proper methods of irrigation. The water should not be run too close to the trees and care should be exercised not to allow the water to stand in any part of the orchard for any great length of time.

SUMMARY

Crown rot is caused by an infectious fungous disease occurring on the crowns and roots of the California black walnuts, *Juglans californica* and *J. hindsii*. It may spread from the black walnut rootstock to the trunk of the English walnut, *J. regia*, where the disease rapidly develops, as the black walnut crown becomes severely infected and is practically girdled.

Evidence is convincing that crown rot of walnut is caused by a species of Phytophthora, provisionally determined as *Phytophthora cactorum*.

The difficulty of isolating this pathogene from the California black walnut is probably due to a toxic substance exuding from the cut

tissues. The conventional methods for showing pathogenicity by satisfying Koch's rules of proof had to be modified for this reason.

Artificial inoculations have caused lesions on the following species of Juglans: *californica, hindsii, nigra, mandshurica, regia, sieboldiana, major, pyriformis, insularis,* and the Paradox hybrid *californica* × *regia*. The aerial lesions caused by artificial inoculation usually become inactive with the coming of the hot weather, and many never again increase in size.

The inoculations made during the years 1923 to 1925 are shown in Tables 1 and 3. Those made in old southern California black walnut trees were the largest in size, with much black exudate. During a year's development, lesions 6 to 8 feet long girdled trunks as large as 8 inches in diameter. The lesions on the northern California black walnut were never so large as those on the southern California black walnut, being usually less than 6 inches in their greatest diameter. English walnut trunks and limbs can be artificially infected, but the lesions from artificial inoculation on healthy trees are never large, usually not more than 6 to 8 inches in diameter.

The inoculations for 1929 were made in seedling trees of Juglans in tissue 50 to 100 mm. in diameter and gave lesions with a radius of 5 to 125 mm. The results showed infection as follows: *Juglans regia*, 90 per cent; *J. californica*, 82 per cent; and *J. hindsii*, 75 per per cent.

It appears that the crown-rot problem for new plantings can be solved by using a resistant stock, probably some variety of *Juglans regia*. Proper use of the irrigation water, with the control of flood water, should greatly aid in crown-rot control for orchards now planted. Exposing the crown by the removal of the dirt has been effective in checking the disease in infected trees. Tree surgery should be employed only in those cases that do not respond to treatment.

LITERATURE CITED

(1) Barrett, J. T., and Smith, C. O.
1926. walnut tree crown rot; its development and suggested treatment. Diamond Walnut News 8 (5): 4–5, 7, illus.

(2) Braucher, O. L.
1930. field trials in crown rot control. that success is based on keeping the tree crowns dry. Diamond Walnut News 12 (4): 9.

(3) Cookson, I. C.
1929. an account of a crown rot of english walnut trees in victoria. Roy. Soc. Victoria, Proc. (n. s.) 42: 5–26, illus.

(4) Dufrénoy, J.
1925. la maladie de l'encre du châtigier. Ann. Off. Agr. Rég. Sud-Ouest, France 10: 79–96, illus.

(5) Fawcett, H. S.
1915. the known distribution of pythiacystis citrophthora and its probable relation to mal di gomma of citrus. Phytopathology 5: 66–67.

(6) ———
1915. melaxuma of the walnut, "juglans regia." (a preliminary report.) Calif. Agr. Expt. Sta. Bul. 261, p. [133]–148, illus.

(7) ———
1916. a pythiacystis on avocado trees. Phytophatholoy 6: 433–435.

(8) ———
1923. gum diseases of citrus trees in california. Calif. Agr. Expt. Sta. Bul. 360, p. 370–423, illus.

(9) Gioda, A.
1928. si puo vincere il male dell'inchiostro? Cultivatore Anno 74 (25): 198–203, illus.
(10) Merrill, E. D.
1929. plant pathology. Calif. Agr. Expt. Sta. Ann. Rpt. 1927–28: 40–44.
(11) Petri, L.
1928. di un nuovo metodo di cura del mal dell'inchiostro del castagno. R. Staz. Patol. Veg. [Rome], Bol. Mens. (n. s.) 8: 339–356, illus.
(12) Smith, E. H.
1915. pythiacystis infection of deciduous nursery stock. Phytopathology 5: 317–322, illus.
(13) Smith, R. E., Ramsey, H. J., Smith, E. H., Babcock, E. B., and Smith, C. O.
1907. the brown rot of the lemon. Calif. Agr. Expt. Sta. Bul. 190, 72 p., illus.
(14) ——— Smith, C. O., and Ramsey, H. J.
1912. walnut culture in california. walnut blight. Calif. Agr. Expt. Sta. Bul. 231, p. [119]–398, illus.
(15) ——— and Smith, E. H.
1906. a new fungus of economic importance. Bot. Gaz. 42: 215–221, illus.
(16) ——— and Smith, E. H.
1925. further studies of pythiaceous infection of deciduous fruit trees in california. Phytopathology 15: 389–404, illus.

THE SOIL VERSUS THE SOLUTION METHOD AS A MEANS OF STUDYING BACTERIAL ACTIVITIES IN SOIL[1]

By J. E. GREAVES, *Professor of Bacteriology and Biochemistry*, and H. C. PULLEY, *Instructor and Assistant in Bacteriology, Department of Bacteriology and Physiological Chemistry, Utah Agricultural Experiment Station*[2]

INTRODUCTION

The biological decomposition of proteinaceous materials as it occurs in ammonification and nitrification in the soil is a dynamic process. There is not only a constant production of ammonia and of nitric acid but the ammonia is continually being transformed into nitric acid and both products are being synthesized into the bacterial proteins. In addition to these transformations there are constantly going on in the soil many other poorly understood side reactions. It is therefore evident that these processes, as they occur in the soil, are reactions which may reach equilibrum but which never go to completion. The bacteriological methods as used to-day merely measure the accumulation of ammonia or of nitric acid present at a specific moment. It therefore seemed important to determine when this measurement should be made in order to obtain the greatest accumulation of products and at what period the greatest differences are manifest between various soils. The natural processes occurring in the soil were studied both in soil and in solution and the results compared.

COMPOSITION OF SOILS USED

The work was conducted on four different soils: The college farm soil obtained just east of the plant industry building on the campus; Corinne soil; Benson soil; and Richland Acres soil.

The physical analyses of these soils, as determined by the Yoder[3] elutriator, are given in Table 1.

TABLE 1.—*Physical composition of soils used in the tests*

Constituent	College farm	Corinne	Benson	Richland Acres
	Per cent	*Per cent*	*Per cent*	*Per cent*
Sand	36.6	17.5	42.5	4.8
Fine sand	30.6	17.3	20.9	29.6
Coarse silt	18.8	28.5	19.0	27.0
Medium	6.0	15.9	3.8	13.1
Fine silt	2.3	5.3	2.3	10.2
Clay	3.5	7.4	6.1	9.4
Moisture, soluble salts, and loss	2.2	8.1	5.4	5.9

The college farm soil is a highly productive calcareous sandy loam, low in organic carbon and nitrogen but otherwise well supplied with the essential elements of plant food. The Benson soil is a sandy

[1] Received for publication Apr. 18, 1931; issued December, 1931. Contribution from the Department of Bacteriology, Utah Agricultural Experiment Station.

[2] Valuable assistance in the analytical work was rendered by Prof. C. T. Hirst, J. Dudley Greaves, and Milton E. Nelson.

[3] YODER, P. A. A NEW CENTRIFUGAL SOIL ELUTRIATOR. Utah Agr. Expt. Sta. Bul. 89, 47 p., illus. 1904.

loam containing more clay that the college farm soil and sufficient alkali carbonates to render it barren. The Corinne and Richland Acres soils are fine sandy loams, the Corinne soil containing sufficient chlorides and the Richland Acres soil sufficient sulphates to render them barren except to a few salt-tolerant plants. Portions of the college farm soils that had received the following treatments were also used:

2 per cent sodium chloride.
2 per cent sodium sulphate.
2 per cent sodium carbonate.
1 per cent sodium chloride plus 1 per cent sodium sulphate.
1 per cent sodium chloride plus 1 per cent sodium carbonate.
1 per cent sodium sulphate plus 1 per cent sodium carbonate.
⅔ per cent each sodium chloride, sodium sulphate, and sodium carbonate.

Consequently there were used in this work (1) the three native alkali soils; (2) the native productive soil; (3) the native productive soil containing salts in the proportions listed above, and (4) each of the preceding soils after they had been leached with water until the major portions of the soluble salts had been removed.

Before the bacteriological analyses were made the hydrogen-ion concentration of each soil was determined by the quinhydrone method. The average pH values for the various soils are given below:

Soil and treatment	pH
College farm:	
Untreated	8.6
Leached	8.5
2 per cent NaCl	8.7
2 per cent NaCl, leached	8.4
2 per cent Na_2SO_4	8.3
2 per cent Na_2SO_4, leached	8.0
2 per cent Na_2CO_3	10.3
2 per cent Na_2CO_3, leached	9.5
1 per cent each of NaCl, Na_2SO_4	8.3
1 per cent each of NaCl, Na_2SO_4, leached	8.4
1 per cent each of Na_2CO_3, NaCl	8.9
1 per cent each of Na_2CO_3, NaCl, leached	8.9
1 per cent each of Na_2SO_4, Na_2CO_3	9.4
1 per cent each of Na_2SO_4, Na_2CO_3, leached	9.5
⅔ per cent each of NaCl, Na_2SO_4, Na_2CO_3	8.8
⅔ per cent each of NaCl, Na_2SO_4, Na_2CO_3, leached	8.9
Corinne	8.5
Leached	8.5
Richland acres	8.9
Leached	9.0
Benson	10.1
Leached	8.7

The pH value of the native productive soil was 8.6. This was slightly reduced by leaching. The addition of the chloride or carbonate of sodium to the soil increased the pH value whereas the addition of sodium sulphate decreased it. The leaching of the soil which contained the single salts decreased the pH, whereas the leaching of the soil containing a combination of salts usually increased the pH. The pH of the Corinne soil was not very different from that of the normal productive soil, nor was it changed by leaching. The Richland Acres soil had a higher alkalinity, which was further increased by leaching. The Benson soil had an alkalinity approximately equal to the productive soil to which had been added 2 per cent of sodium carbonate. This was greatly reduced by leaching. It is apparent that

there was an appreciable variation in the pH values of the different soils, depending upon the soluble salt that they contained.

TABLE 2.—*Replaceable bases, expressed as milligram equivalents per 100 gm. of soil, in soils used for tests when differently treated*

Soil and treatment	Calcium	Magnesium	Sodium	Potassium	Equivalent of NH_3 required
College farm	23.7	4.5	8.7	0.4	10.6
College farm, leached	21.8	2.1	.6	.4	11.6
NaCl added, leached	24.1	2.6	1.2	.6	10.1
Na_2SO_4 added, leached	26.2	3.8	1.0	.6	11.1
Na_2CO_3 added, leached	21.9	2.7	8.7	.2	8.2
NaCl, Na_2SO_4 added, leached	27.0	2.5	8.7	.1	9.4
Na_2SO_4, Na_2CO_3 added, leached	18.7	2.4	8.7	.3	8.5
Na_2SO_4, NaCl, Na_2CO_3 added, leached	25.4	3.4	1.5	.8	9.6
Corinne, leached	9.1	5.6	5.9	1.0	8.6
Richland Acres, leached	16.2	26.5	7.6	.8	10.2
Benson, leached	17.4	23.6	4.2	1.6	5.5

From the data shown in Table 2 one may gain an idea of the replaceable bases in the various soils. The replaceable bases in the leached soils were in all cases lower than in the nonleached soils. The leaching had carried from the soils appreciable quantities of sodium, potassium, calcium, and magnesium.[4] Yet the leached normal soil had a more active microflora and was more productive than the normal nonleached soil.[5] The soil to which sodium carbonate had been added and which had afterwards been leached had lost considerable of its replaceable calcium, magnesium, and potassium. The native alkali soils were lower in calcium but higher in the other replaceable bases than was the native fertile soil. It is evident, therefore, that these soils varied greatly—chemically, physically, and biologically. The differences in their bacterial activities and productivity were readily measured by the ordinary means.[5]

AMMONIFICATION IN SOLUTION

Preliminary studies were made on the rate of ammonia accumulation in solution. This was done by seeding 1-gm. portions of each of the soils into 100 c. c. portions of peptone solution. These were incubated at from 28° to 30° C. Aliquot portions of the solution were removed daily under aseptic conditions in order not to contaminate the remainder of the media. The ammonia was determined in this sample and the quantity calculated in the original medium. The average results for the various soils for 11-day periods are given in Table 3.

Only small quantities of ammonia accumulated in the media during the first 24 hours, yet there was an appreciable variation, depending upon the specific soil used. Usually at the end of 24 hours there were greater accumulations of ammonia where the leached soil was used than where the unleached soils were used. The mere leaching of the normal soil produced a measurable variation in ammonia accumulation at first but not at the end of 24 hours.

[4] GREAVES, J. E., HIRST, C. T., and LUND, Y. THE LEACHING OF ALKALI SOILS. Soil Science, 16: 407–426. 1923.

[5] GREAVES, J. E. THE MICROFLORA AND THE PRODUCTIVITY OF LEACHED AND NONLEACHED ALKALI SOIL Soil Science, 23: 271–302. 1927.

TABLE 3.—*Milligrams of ammonia found in peptone after various incubation periods*

[The media had been inoculated with soil containing various soluble salts]

Soil and treatment	Milligrams of ammonia produced in—										
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days
Normal	3.9	21.7	26.5	28.9	52.0	59.8	52.4	58.4	51.5	52.8	47.0
Normal, leached	5.8	20.8	25.9	29.6	50.5	60.6	46.7	55.9	53.7	55.3	46.5
NaCl	3.5	20.8	22.6	28.6	50.8	56.3	57.4	55.6	59.7	55.3	53.2
NaCl, leached	3.3	21.7	22.9	31.6	52.8	63.3	66.1	59.6	58.3	58.0	55.2
Na_2SO_4	3.7	18.4	25.9	32.2	53.0	56.3	56.1	60.9	53.7	54.6	57.8
Na_2SO_4, leached	2.5	19.1	22.2	34.6	48.5	58.0	52.7	60.2	48.3	52.0	46.5
Na_2CO_3	1.1	11.7	16.4	26.2	49.2	62.0	60.1	66.2	57.0	60.6	60.5
Na_2CO_3, leached	2.1	23.7	25.9	37.2	64.5	64.3	70.7	61.6	59.0	57.3	57.2
NaCl, Na_2SO_4	2.5	18.4	27.6	37.9	55.2	50.0	63.4	58.2	51.7	55.3	51.2
NaCl, Na_2SO_4, leached	4.6	24.6	27.6	31.9	60.5	58.6	56.7	56.9	52.3	54.6	52.5
NaCl, Na_2CO_3	2.9	16.2	18.6	27.2	58.5	52.6	70.1	57.6	55.7	52.0	46.5
NaCl, Na_2CO_3, leached	4.9	22.8	20.4	27.2	60.5	57.3	63.4	58.2	63.0	59.3	49.8
Na_2SO_4, Na_2CO_3	1.6	24.6	25.0	34.2	61.8	62.0	60.7	54.9	59.7	55.3	47.8
Na_2SO_4, Na_2CO_3, leached	4.3	22.4	29.6	26.6	65.2	54.6	58.1	58.9	51.7	59.3	46.5
NaCl, Na_2SO_4, Na_2CO_3	2.7	14.2	29.6	27.9	59.8	60.0	54.1	52.9	55.0	45.3	45.2
NaCl, Na_2SO_4, Na_2CO_3, leached	4.2	24.2	30.6	26.9	53.6	62.0	52.7	51.6	49.7	44.0	45.8
Corinne	2.5	15.7	25.6	26.4	63.8	54.6	49.4	59.6	47.0	48.6	43.2
Corinne, leached	4.6	20.2	25.9	27.2	67.8	62.6	56.7	54.9	55.0	60.0	48.5
Richland Acres	2.5	18.4	34.6	30.6	65.2	59.3	57.4	47.6	50.3	45.3	46.5
Richland Acres, leached	4.6	26.6	35.9	30.9	66.5	58.0	60.4	61.6	58.3	47.3	50.5
Benson	1.3	3.1	9.9	14.9	33.8	35.3	41.4	34.2	36.7	42.6	33.2
Benson, leached	2.0	18.2	34.9	27.6	61.8	64.3	52.7	56.2	49.7	52.0	43.2

Considerably more ammonia had accumulated by the end of the second day. However, the variation in the different soils was in the same order, differing only in magnitude. The accumulations of ammonia during the third and fourth days were not as rapid as on the second day. The rate of production reached its height between the fourth and fifth days. After this period there was a slowing down of the reaction. In a few cases there was an actual loss of ammonia during the latter part of the incubation period. The variation due to different soils was in the same order during the first seven days. Beyond this period the results approach a constant. The widest variation in ammonia accumulation in the various soils is found when the determinations are made between the fourth and seventh days. The accumulation of ammonia was greater on the seventh day than on the fourth. Other than the greater accumulation of ammonia, nothing is to be gained by taking the longer incubation period. In each incubation period is manifested the influence of treatment; hence, any of the early periods may be used in studying ammonification provided determinations made only with the same incubation period are compared. It is certain that the ammonifying ferments are not destroyed in soils by as much as 2 per cent of alkali even after long periods, as when the soluble salts are removed or diluted the ferments become active.

NITRIFICATION IN SOLUTION

A study similar to that reported above was made of the nitrifying powers of the same soils. The technic was the same for the ammonification series except that 0.1 per cent of sodium nitrite was used as the substrate to be nitrified. The average results for the sulphate-carrying soils are given in Table 4.

TABLE 4.—*Milligrams of nitric nitrogen found in nitrite media after various incubation periods*

[The media had been inoculated with soil modified by adding sulphates alone and in combination with other salts]

Soil and treatment	Milligrams of nitric nitrogen produced in—											
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Normal	1.5	1.0	1.8	1.5	3.2	3.3	2.7	2.8	2.5	2.7	4.9	8.9
Normal, leached	1.1	2.0	2.2	1.7	3.7	3.4	3.3	3.6	2.3	3.7	4.9	8.6
Na_2SO_4	1.0	1.4	3.1	3.5	2.5	3.3	3.4	3.0	2.0	2.8	2.5	4.1
Na_2SO_4, leached	.9	1.8	2.3	4.4	3.9	3.4	2.6	3.0	2.1	3.7	3.7	6.0
NaCl, Na_2SO_4	.8	1.6	2.4	2.0	2.8	4.3	3.2	2.8	1.9	2.2	2.5	2.2
NaCl, Na_2SO_4, leached	.8	1.6	2.2	2.1	2.2	3.9	3.4	3.0	2.9	3.9	4.5	5.8
Na_2CO_3, Na_2SO_4	1.1	1.4	2.5	4.1	2.9	3.5	3.4	3.1	2.8	3.5	4.4	6.4
Na_2CO_3, Na_2SO_4, leached	1.1	1.9	2.0	3.4	2.1	2.9	6.3	6.0	4.9	6.5	9.5	20.5
NaCl, Na_2SO_4, Na_2CO_3	1.0	1.2	2.6	1.6	3.1	2.7	5.4	4.8	2.6	3.4	7.0	13.6
NaCl, Na_2SO_4, Na_2CO_3, leached	1.1	1.4	2.3	1.5	2.0	2.4	6.4	3.1	2.8	3.2	4.3	6.5

Soil and treatment	Milligrams of nitric nitrogen produced in—										
	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days	29 days	36 days
Normal	12.0	25.5	33.7	41.0	50.0	84.0	84.0	95.0	100.0	104.0	114.0
Normal, leached	16.1	23.3	29.1	41.8	53.0	71.0	89.5	92.5	106.0	103.0	99.0
Na_2SO_4	4.8	8.6	9.5	31.7	51.0	59.0	78.0	95.5	94.0	78.0	95.0
Na_2SO_4, leached	10.0	15.0	17.7	38.0	48.0	53.0	95.0	100.0	97.0	92.0	88.0
NaCl, Na_2SO_4	3.0	2.7	2.3	3.9	3.2	7.4	31.0	50.0	81.0	93.0	97.0
NaCl, Na_2SO_4, leached	14.5	18.0	23.3	37.5	42.5	54.5	69.0	90.0	87.5	98.0	88.0
Na_2CO_3, Na_2SO_4	16.5	21.8	24.7	53.0	62.5	81.0	88.0	103.0	95.5	95.0	95.0
Na_2CO_3, Na_2SO_4, leached	32.8	43.3	44.5	81.5	72.0	79.0	88.0	102.0	95.5	82.0	96.0
NaCl, Na_2SO_4, Na_2CO_3	34.5	50.0	69.0	102.5	85.0	93.5	92.0	94.0	92.5	93.0	93.0
NaCl, Na_2SO_4, Na_2CO_3, leached	19.0	22.1	30.0	68.0	76.0	96.5	96.0	98.0	91.5	91.0	95.0

The accumulation of nitrates during the first 11 days was extremely slow, after which there was an acceleration of the reaction. The rate of the reaction varied widely, depending upon the soil. Where the inoculation had been made with soil containing the three salts, the maximum accumulation of nitric nitrogen was reached between the fifteenth and sixteenth days. After this period there was a decrease in the nitrate content of this solution. This may have been due more to its transformation into protein nitrogen than to a slowing down of the reaction. The corresponding leached soil reached its maximum between the nineteenth and twentieth days. During the first 18 days the soil containing sodium chloride and sodium sulphate caused little accumulation of nitrates, but during the succeeding few days the gains were as great as in the other soils. If one were attempting to measure maximum accumulation of nitric nitrogen, the incubation period should be taken as 21 days, or even longer; whereas, if one wishes to measure the relative speed of nitric nitrogen accumulation and hence the nitrifying efficiency of the various alkali soils it is better to use an incubation period of 15 days.

Two per cent of sodium sulphate in this soil was sufficient to retard the activity but not to destroy the nitrifying ferments, for when conditions were made appropriate the nitrifying ferments commenced to work.

The average results for the chloride-carrying soils are given in Table 5.

TABLE 5.—*Milligrams of nitric nitrogen found in nitrite media after various incubation periods*

[The media had been inoculated with soil modified by adding chlorides alone and in combination with other salts]

Soil and treatment	Milligrams of nitric nitrogen produced in—											
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Normal	1.5	1.0	1.8	1.5	3.2	3.3	2.7	2.8	2.5	2.7	4.9	8.9
Normal, leached	1.1	2.0	2.2	1.7	3.7	3.4	3.3	3.6	2.3	3.7	4.9	8.6
NaCl, leached	.9	1.7	2.7	1.9	3.8	3.6	2.8	3.2	2.3	2.7	3.9	6.9
NaCl, Na_2SO_4	.8	1.6	2.4	2.0	2.8	4.3	3.2	2.8	1.9	2.2	2.5	2.2
NaCl, Na_2SO_4, leached	.8	1.6	2.2	2.1	2.2	3.9	3.4	3.0	2.9	3.9	4.5	5.8
NaCl, Na_2CO_3	1.3	1.6	2.6	1.9	2.4	3.5	4.0	2.8	1.9	2.2	2.6	2.5
NaCl, Na_2CO_3, leached	.9	1.1	2.6	3.2	3.7	4.3	3.5	3.9	4.2	6.7	13.0	24.2
NaCl, Na_2SO_4, Na_2CO_3	1.0	1.2	2.6	1.6	3.1	2.7	5.4	4.8	2.6	3.4	7.0	13.6
NaCl, Na_2SO_4, Na_2CO_3, leached	1.1	1.4	2.3	1.5	2.0	2.4	6.4	3.1	2.8	3.2	4.3	6.5

Soil and treatment	Milligrams of nitric nitrogen produced in—										
	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days	29 days	36 days
Normal	12.0	25.5	33.7	41.0	50.0	84.0	84.0	95.0	100.0	104.0	114.0
Normal, leached	16.1	23.3	29.1	41.8	53.0	71.0	89.5	92.5	106.0	103.0	99.0
NaCl, leached	12.1	20.5	23.8	40.8	50.0	67.0	93.5	98.0	95.0	98.0	98.0
NaCl, Na_2SO_4	3.0	2.7	2.3	3.9	3.2	7.4	31.0	50.0	81.0	93.0	97.0
NaCl, Na_2SO_4, leached	14.5	18.0	23.3	37.5	42.5	54.5	69.0	90.0	87.5	98.0	88.0
NaCl, Na_2CO_3	3.1	2.4	1.8	2.9	5.1	3.1	5.4	10.1	58.0	90.0	91.0
NaCl, Na_2CO_3, leached	48.8	55.5	77.0	110.0	92.0	93.0	96.5	92.0	94.0	95.0	95.0
NaCl, Na_2SO_4, Na_2CO_3	34.5	50.0	69.0	102.5	85.0	93.5	92.0	94.0	92.5	93.0	93.0
NaCl, Na_2SO_4, Na_2CO_3, leached	19.0	22.1	30.0	68.0	76.0	96.5	96.0	98.0	91.5	91.0	95.0

Little nitric nitrogen accumulated during the first 11 days. All solutions contained approximately the same quantities. After this period there was a wide variation and in most cases a rapid accumulation. By the sixteenth day the leached sodium carbonate-sodium chloride soil had accumulated 110 parts per million of nitric nitrogen, whereas the corresponding unleached soil had accumulated only 3 parts per million. It was not until the twentieth day that this soil-inoculated medium commenced to accumulate much nitric nitrogen, but by the twenty-ninth day the accumulation was about as great as in the other soils. It is evident also in this series that the shorter incubation period of 15 days should be used if one wishes to determine the influence of soluble salts on nitrification by the solution method.

The average results for the carbonate-treated soils are given in Table 6.

TABLE 6.—*Milligrams of nitric nitrogen found in nitrite media after various incubation periods*

[The media had been inoculated with soil modified by adding carbonates alone and in combination with other salts]

Soil and treatment	Milligrams of ammonia produced in—											
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Normal	1.5	1.0	1.8	1.5	3.2	3.3	2.7	2.8	2.5	2.7	4.9	8.9
Normal, leached	1.1	2.0	2.2	1.7	3.7	3.4	3.3	3.6	2.3	3.7	4.9	8.6
Na_2CO_3	1.5	2.5	3.5	3.9	3.1	3.4	3.5	3.6	2.0	2.2	2.3	3.0
Na_2CO_3, leached	.9	1.3	2.0	2.0	2.5	3.0	3.8	3.5	2.8	4.7	8.9	22.2
NaCl, Na_2CO_3	1.3	1.6	2.6	1.9	2.4	3.5	4.0	2.8	1.9	2.2	2.6	2.5
NaCl, Na_2CO_3, leached	.9	1.1	2.6	3.2	3.7	4.3	3.4	3.9	4.2	6.7	13.0	24.2
Na_2CO_3, Na_2SO_4	1.1	1.4	2.5	4.1	2.9	3.5	3.4	3.1	2.8	3.5	4.4	6.4
Na_2CO_3, Na_2SO_4, leached	1.1	1.9	2.0	3.4	2.1	2.9	6.3	6.0	4.9	6.5	9.5	20.5
NaCl, Na_2CO_3, Na_2SO_4	1.0	1.2	2.6	1.6	3.1	2.7	5.4	4.8	2.6	3.4	7.0	13.6
NaCl, Na_2CO_3, Na_2SO_4, leached	1.1	1.4	2.3	1.5	2.1	2.4	6.4	3.1	2.8	3.2	4.3	6.5

Soil and treatment	Milligrams of ammonia produced in—										
	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days	29 days	36 days
Normal	12.0	25.5	33.7	41.0	50.0	84.0	84.0	95.0	100.0	104.0	114.0
Normal, leached	16.1	23.3	29.1	41.8	53.0	71.0	89.5	92.5	106.0	103.0	99.0
Na_2CO_3	3.0	3.2	1.8	3.3	2.7	2.8	6.3	3.0	5.0	7.3	28.0
Na_2CO_3, leached	47.5	78.5	84.5	127.0	96.5	95.0	94.5	101.0	96.0	91.0	98.0
NaCl, Na_2CO_3	3.1	2.4	1.8	2.9	5.1	3.1	5.4	10.1	58.0	90.0	91.0
NaCl, Na_2CO_3, leached	48.8	55.5	77.0	110.0	92.0	93.0	96.5	92.0	94.0	95.0	95.0
Na_2CO_3, Na_2SO_4	16.5	21.8	24.7	53.0	62.5	81.0	88.0	103.0	95.5	95.0	95.0
Na_2CO_3, Na_2SO_4, leached	32.8	43.3	44.5	81.5	72.0	79.0	88.0	102.0	95.5	82.0	96.0
NaCl, Na_2CO_3, Na_2SO_4	34.5	50.0	69.0	102.5	85.0	93.5	92.0	94.0	92.5	93.0	93.0
NaCl, Na_2CO_3, Na_2SO_4, leached	19.0	22.1	30.0	68.0	76.0	96.5	96.0	98.0	91.5	91.0	95 0

The soils containing small quantities of sodium carbonate accumulate nitric nitrogen more rapidly than the other soils, but when large quantities of the carbonates are present there is great retardation of the process. The slowest nitrification of the whole series was by the soil containing 2 per cent sodium carbonate, whereas the highest accumulation was by the same soil after leaching. In this series also the maximum variation occurred on the fifteenth day, and by the twenty-first day the variation due to the different salt-treated soils was slight, with the exception of the soil containing 2 per cent sodium carbonate. On the twenty-first day it had accumulated only 5 parts per million of nitric nitrogen; by the end of the thirty-sixth day there were 28 parts per million, thus indicating that the nitrifying ferments are not destroyed by 2 per cent sodium carbonate in soil.

The average results obtained from a study of the three native alkali soils, leached and nonleached, are given in Table 7.

TABLE 7.—*Milligrams of nitric nitrogen found in nitric media after various incubation periods*

[The media had been inoculated with various alkali soils]

Soil and treatment	Milligrams of nitric nitrogen produced in—											
	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	11 days	12 days
Corinne	1.2	1.3	2.1	2.3	4.2	2.4	5.7	2.5	2.1	2.2	2.6	2.5
Corinne, leached	1.8	1.6	2.0	3.1	2.5	2.4	5.2	3.3	2.3	3.0	4.0	4.9
Richland Acres	1.4	1.1	2.7	2.0	2.7	2.3	8.7	3.7	2.7	3.9	5.8	8.2
Richland Acres, leached	1.3	1.2	2.5	3.3	2.6	3.0	4.9	4.4	3.0	4.7	6.4	10.5
Benson	1.4	1.4	2.4	3.3	4.3	3.2	5.3	3.5	2.7	2.5	2.4	4.0
Benson, leached	1.5	1.4	2.3	1.9	2.6	2.7	9.0	5.7	3.1	2.8	3.1	2.8

Soil and treatment	Milligrams of nitric nitrogen produced in—										
	13 days	14 days	15 days	16 days	17 days	18 days	19 days	20 days	21 days	29 days	36 days
Corinne	3.1	2.1	1.8	2.8	2.9	5.1	16.0	27.0	32.0	84.0	94.0
Corinne, leached	9.5	11.2	14.8	37.0	45.5	73.0	93.8	89.0	90.0	96.0	94.0
Richlahd Acres	17.5	19.8	31.3	49.5	57.5	89.5	97.0	100.0	93.5	93.0	90.0
Richland Acres, leached	24.3	25.5	37.8	59.5	67.0	89.5	95.5	92.0	91.0	93.0	90.0
Benson	3.7	4.2	2.1	3.1	2.4	2.5	3.5	2.6	5.0	55.0	91.0
Benson, leached	4.2	3.0	3.0	5.5	3.4	6.9	9.7	13.9	17.4	68.0	93.0

During the first 12 days little nitric nitrogen accumulated in any of the media. However, a rapid accumulation occurred between the twelfth and eighteenth days in the Richland Acres soils, leached and nonleached, and in the Corinne leached soil. The Corinne leached soil accumulated small quantities of nitric nitrogen during the first 18 days, but by the end of the twenty-ninth day the media into which it had been inoculated was carrying about as much nitric nitrogen as did the other media. The Benson leached and nonleached soil caused slight accumulation of nitric nitrogen the first 18 days, but by the thirty-sixth day the accumulation was as great as with the other soils. In the native alkali soils the greatest variation of nitric nitrogen in the solutions occurred later than the fifteenth day.

It is evident from the results on all the soils that the greatest variation in accumulation of nitric nitrogen in the solutions inoculated with the various soils occurs between the 15 and 18 days. By the thirty-sixth day the quantity of nitric nitrogen in the media treated with the various soils approaches a constant. If one uses the shorter incubation periods, it is evident that there is a wide variation in the nitric nitrogen accumulation in liquid media. This varies with the specific soil which has been used as the inoculum. It is further evident that soluble salts stimulate or retard nitrification as measured by this method, the extent depending upon the specific salt, its combination with other salts, and its concentration. Even large quantities of the soluble salts may occur in soils over long periods without destroying the nitrifying ferments. The salts inhibit their actions and when diluted the action of the nitrifying ferments is manifest.

AMMONIFICATION IN SOIL VERSUS IN SOLUTION

The ammonifying powers of the various soils were determined both by the soil and the solution methods. The technic for the solution method was the same as that previously given. The incubation period was seven days. The substrate to be ammonified was peptone. The tests in soil were made by mixing 2 gm. of dried blood with 100 gm. of soil in tumblers, making the moisture up to 18 per cent, covering with Petri dishes and incubating for seven days at 28° to 30° C. The ammonia was then determined in the regular way. The average results for both the solution and the soil methods are given in Figure 1.

The native productive soil was a highly calcareous loam which became more productive on leaching. The addition of soluble salts to it had rendered it barren. The three native alkali soils were barren. The leaching of the soils rendered a few productive, and in

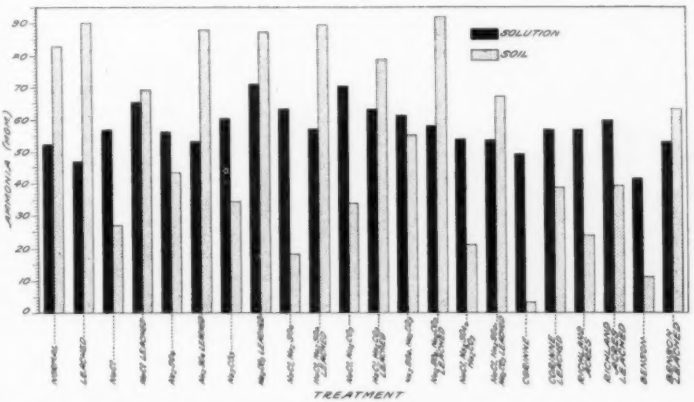


FIGURE 1.—Milligrams of ammonia found in soil and in liquid media inoculated with the same soils

some cases the leached soils produced larger crops of crimson clover than the native nonalkali soil. The increase usually occurred where the sulphates had been added and was due in part to the addition of sulphur to this soil low in sulphur.[6] There is a close correlation between the productivity of the soils and their ammonifying powers, as measured by the soil-tumbler method. If the ammonia accumulations in the soil are compared with those in the solution, a direct correlation is found in only three cases; these are leached soils. The soluble salts depress in the soil; where diluted, however, as in the solution, they often stimulate. After the salts have been leached from the soil and the soil inoculated into the solution the concentration of the salt is usually too low to stimulate. The Corinne soil, leached and nonleached, is nonproductive. The nonleached soil produces little ammonia in the soil, whereas in the solution there is a large accumulation. The leaching increases the accumulation both in the soil and in the solution. One might conclude from the results obtained

[6] GREAVES, J. E., and GARDNER, W. IS SULFUR A LIMITING FACTOR OF CROP PRODUCTION IN SOME UTAH SOILS? Soil Sci. 27: 445-457, illus. 1929.

on this soil by the soil-tumbler method that the ammonifying organisms are absent, which is certainly erroneous, as is seen by an examination of the results obtained by the solution method. Consequently, it may be concluded that where one desires to gain an insight into the relative productivity of a soil the soil-tumbler method is greatly to be preferred to the solution method for testing the soil. If one desires to determine the presence or absence of the ammonifying ferments the solution method is to be preferred to the soil-tumbler method.

NITRIFICATION IN SOIL VERSUS IN SOLUTION

Nitrification both in soil and in solution was determined on all the soils previously described. The technic was the same as that described under ammonification except that in solution the substrate to be nitrified was sodium nitrite and the incubation periods in the solu-

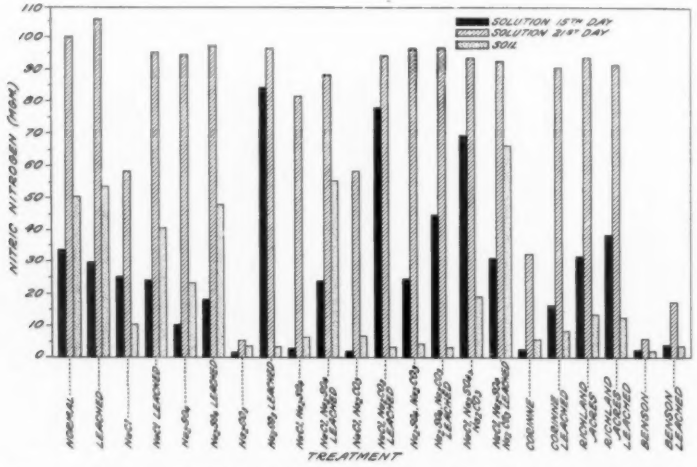


FIGURE 2.—Milligrams of nitric nitrogen found in soil and liquid media inoculated with the same soil. The incubation periods were 15 and 21 days for the solutions and 21 days for the soil

tions were 15 and 21 days, whereas in the soil an incubation period of 21 days was used. The average results for soil and solution are given in Figure 2.

The determinations made in soil, with four exceptions, yielded greater accumulations of nitric nitrogen in the leached than in the nonleached soil, the exceptions being where the carbonate was present. The carbonate left the leached soil in a bad physical condition, the soil structure having been broken down. There was in many cases an appreciable stimulation of nitrification due to the soluble salts. From these results one might erroneously conclude that sodium carbonate had destroyed the nitrifiying ferments. However, their activities were only inhibited by the soluble salts present, as is seen from the results obtained in solution. Hence, in dilute solutions they often act as stimulants. The stimulation may be real or apparent: (1) The salts may accelerate the activities of the

ammonifers; consequently, ammonia would be produced more rapidly for the use of the nitrifers. This may occur where the dried blood is the substrate decomposed but not where sodium nitrite was used. (2) They may depress denitrification or the transformation of the nitrates into protein nitrogen. Inasmuch as tests of these processes indicate that they are accelerated and not retarded by weak concentrations of these soluble salts it is not probable that the accumulation is due to this factor. (3) They may stimulate more rapid nitrification. This last possibility appears to be the correct interpretation of the data. The accumulation of nitric nitrogen in the solution during 21 days, with three exceptions, is greater in the leached than in the nonleached soils. In the soil there is a stimulation in three cases; in the solution in one case.

On the fifteenth day of incubation all except two of the solutions which had been seeded with the leached soils showed a gain in nitric nitrogen over the nonleached soils. The spread between the various soils is more pronounced on the fifteenth day than it is on the twenty-first. Consequently, it may be concluded from the study of nitrification in alkali soils that where the solution method is used and the substrate is sodium nitrite an incubation period of 15 days should be taken. Under these conditions, results comparable to those obtained in soil with dried blood as the substrate and an incubation period of 21 days are obtained. However, if one is testing for the presence of the nitrifying ferments, it is better to use the solution method with an incubation period of 21 days; otherwise, one may conclude that the alkalies in some instances have destroyed the nitrifying ferments. It is evident from the results obtained with an incubation period of 21 days that the nitrifying ferments will tolerate over long periods as much as 2 per cent of sodium chloride, sodium sulphate, or sodium carbonate in a soil.

NITROGEN FIXATION IN SOLUTION VERSUS IN SOIL

The nitrogen-fixing powers of the soil were determined in both soil and solution. In soil the determinations were made by adding 2 per cent mannite to soil in tumblers. The moisture was held at approximately 20 per cent. The cultures were incubated at 28° to 30° C. for 21 days and the nitrogen determined.

In solution, the determinations were made by seeding 1-gm. portions of the various soils into 100 c. c. portions of Ashby solution containing 2 per cent mannite. These cultures were incubated for 21 days and the nitrogen determined.

The average results for soils and solutions are given in Figure 3.

There is little correlation between the results obtained in solution and those obtained in soil. In solution the native leached soil and the sodium-sulphate leached soils gained more nitrogen than did the native soil. All soils showed slight gains in nitrogen, thus demonstrating the presence of nitrogen-fixing organisms. With few exceptions the leached soils gained larger quantities of nitrogen than did the nonleached soils. In most cases the fixation was greater in the soil than in the solution; hence, care should be used in comparing nitrogen fixation as measured by the two methods. Many of the salts stimulate fixation in the soil, and in a few cases, as for instance in the Benson soil, the gain is extraordinary; this property has been repeatedly observed in a study of this soil. The Richland Acres soil makes

no gains in nitrogen when incubated directly, but when inoculated into Ashby solution gains appreciable quantities of nitrogen.

SUMMARY

An extensive bacteriological study was made on soils varying widely in physical, chemical, and biological properties. One of the soils used was a highly productive calcareous loam. This was employed in its native condition and also after it had been made unproductive by the addition of three salts, both singly and in different combinations of twos and threes. Still other samples of the native and variously treated soils were leached and then used in the experimental work. With these artificially prepared soils were used three native alkali soils, both leached and unleached. The hydrogen-ion concentration of the different soils varied from pH 8.0 to 10.3; the replaceable bases—calcium, magnesium, sodium, and potassium—varied

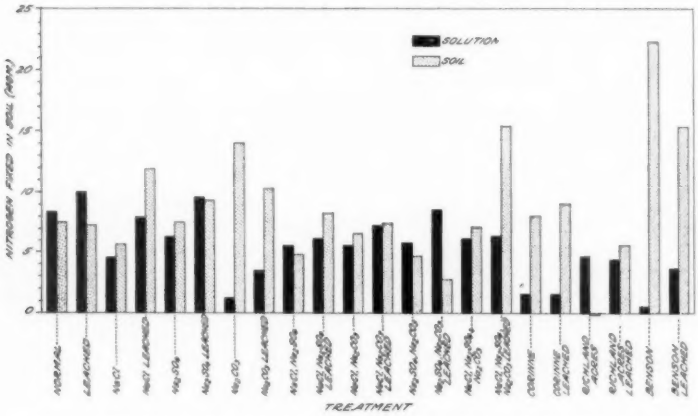


FIGURE 3.—Milligrams of nitrogen fixed in soil and in Ashby media inoculated with the same soils. The incubation periods were 21 days

widely in the different soils and to a lesser extent in the variously treated soils.

When the soils were inoculated into peptone solution the production of ammonia varied with the specific soils and the incubation period. It was slow, then rapid, followed by a slowing down of the process and a decrease in ammonia, probably due to its transformation into protein nitrogen. The ammonia accumulation varied, depending upon the specific soil, its treatment, and the incubation period. It would appear that an incubation period of from one to seven days may be used; however, results obtained only with similar incubation periods are comparable.

The greatest accumulation of nitric nitrogen occurred between the nineteenth and twenty-first days, but the greatest variation in nitric nitrogen accumulation occurred on the fifteenth day. Consequently, if one wishes to obtain the maximum accumulation of nitric nitrogen in solutions an incubation period of 21 days should be used, but if one

wishes to learn the relative rate of nitric nitrogen accumulation in alkali soils an incubation period of 15 days should be used.

By using an incubation period of 15 days one gets a fair correlation between solution and soil method. One may at times conclude from results obtained that soluble salts in concentrations of 2 per cent destroy the nitrifying ferments, a conclusion that is shown to be erroneous when the results obtained in solutions are examined. The ammonifying, nitrifying, and nitrogen-fixing ferments withstand 2 per cent of sodium sulfate, sodium carbonate, or sodium chloride in soil for long periods.

All soils studied showed gains in nitrogen, both by the soil and by the solution methods. However, the gains were usually greater when the determinations were made by the soil-tumbler method than when they were made by the solution method.

CHANGES IN THE CHEMICAL COMPOSITION OF MANGELS AND RUTABAGAS DURING STORAGE[1]

By K. S. MORROW, *Assistant Dairy Husbandman*, R. B. DUSTMAN, *Agricultural Chemist*, and H. O. HENDERSON, *Dairy Husbandman, West Virginia Agricultural Experiment Station*

INTRODUCTION

Mangels, rutabagas, and other root crops have long been grown and fed as a source of succulence for farm animals, especially dairy cows. The storage period of these crops, ranging from one to several months, generally extends through a portion of the winter season. Changes occurring in the physical appearance of the roots as evidenced by shriveling and withering are probably affected by the length of storage and the conditions under which they are kept. An investigation of the varietal differences in the chemical composition of certain mangels and rutabagas grown at this station for dairy-feeding purposes afforded an opportunity to study also the relative changes occurring during the storage of these same root crops as shown by chemical analyses.

WORK OF OTHER INVESTIGATORS

A difference of opinion has existed regarding the actual effect of storage on the feeding value of root crops. Miles (*6*)[2] in 1841 stated that sugar beets should be stored for some time before being fed in order to prevent the action of certain toxic properties. Voelcker (*10*) in 1859 suggested the importance of determining by precise experiments at what time stored roots acquire their maximum feeding properties, and Drummond, Channon, and their coworkers (*3*) recently mentioned a belief among English farmers that mangels have a higher feeding value in early spring than they have immediately after they are pulled in early winter. In contrast to these opinions are those of Miller (*7*, *pt. I*), who doubted the advisability of prolonged storage as a means of increasing digestibility of mangels because of the small amount of crude fiber present in them, and of Shutt (*9*), who stated that an actual loss occurred due to fermentative changes that destroyed some of the sugar.

The chemical changes that take place in roots during storage have received but little study. The loss of moisture and subsequent increase in dry matter are the changes most frequently mentioned. Voelcker (*10*) analyzed a single mangel after two years of storage and found that even after so prolonged a period it still retained 92.25 per cent moisture. No analysis of the root at the time of storage was given for comparison. The dry matter showed 15.87 per cent inorganic salts and 1.67 per cent insoluble mineral matter, which was interpreted as evidence of a loss in feeding substances due to fermentation of dry organic matter which escaped in a gaseous state. Pagnoul

[1] Received for publication May 8, 1931; issued December, 1931. Scientific Paper No. 101 of the West Virginia Agricultural Experiment Station.

[2] Reference is made by number (italic) to Literature Cited, p. 930.

(*8*) reported a loss of 25 per cent of crystallizable sugar by fermentation when sugar beets were stored for a period of three months (April to June).

Losses of nutrients expressed in percentage of original content, for mangels stored from October to July, were reported by Miller (*7*), as follows: Dry matter 16.03, organic matter 19.08, and sugar 20.56. A single mangel stored for one year showed a percentage loss of water of 8.44, organic matter 59.90, sugar 87.50, crude fiber 20.30, and digestible protein 49.30. Shutt (*9*), reporting on three varieties of mangels analyzed in October, January, and March, stated that the nitrates resulting from high manuring with soluble nitrogenous fertilizer tended to disappear during the storage of roots, whereas nonalbuminoid nitrogenous compounds as a rule increased with storage, especially during the winter months. A recent study by Benoy (*2*) showed a respiratory loss of 0.807 gm. of carbon per 100 gm. dry weight in beets held at 30° C. for 26 hours after cutting.

METHODS OF EXPERIMENTATION

Beginning with the season of 1925 and continuing for a period of three years, analyses are available that show the changes in the composition of mangels and rutabagas during storage for each of the years studied. Five sets of analyses were made each year, representing samples obtained approximately one month before harvest, at the time of harvest or the beginning of the storage period, and at intervals of one month each for three months after storage. The date of harvest ranged from October 20 to November 1. In sampling root crops of this nature one has a choice of two general methods of procedure (1) sampling of the same individual roots with some type of boring instrument at successive periods, with consequent liability to changes due to mutilation, and (2) sampling of different individual roots at successive periods, with liability to individual variation. Probably either method, carefully performed, would yield trustworthy results. In this experiment the first method was adopted as being the more suitable one. Eight to ten representative plants of each variety were selected in the field and sampled, while still growing, by the use of a cheese trier, a single diagonal boring being taken from each root. A similar method of sampling was employed throughout the season, the same roots being used at each period. Such borings may not accurately represent the average composition of the entire roots, but it is not necessary that they should. If the borings are comparable from period to period any trend in nutrient changes will be quite apparent irrespective of whether the percentage figures themselves are slightly above or slightly below the absolute values for the entire root. The sampled roots were harvested at the same time as the remainder of the crop, they were identified by tags, and stored in boxes in a root cellar along with the remainder of the season's crop. The root cellar, which had a dirt floor and concrete walls and ceiling, was situated under one end of the cow stable. Ventilation was not provided except as the entrance door was allowed to remain open during the cutting and removal of roots from the cellar.

As soon as the sample borings were removed from the roots they were placed in air-tight glass containers and taken to the laboratory for analysis. Here the containers were weighed, emptied, again

weighed, and the contents run through a small meat chopper and caught in a 12-inch evaporating dish. The material in the dishes was then mixed, spread in thin layers, and brought to air dryness with the aid of an electric fan, after which it was returned to the original containers and weighed. Finally the samples were ground fine in an iron mortar and the regular analysis completed. Determinations for moisture, protein, fat, crude fiber, ash, and nitrogen-free extract were made by the customary methods of the Association of Official Agricultural Chemists (*1*).

Eight varieties of mangels and six varieties of rutabagas are represented, in a few cases a variety being grown but a single year. A total of 1,050 analyses were used in the study.

EXPERIMENTAL RESULTS

CHANGES IN MOISTURE CONTENT

One of the most marked changes in the composition of root crops during storage is a continuous loss of moisture. Figure 1 presents graphically the change in the moisture content of mangels and rutabagas for a period entending from one month before harvest to three months after harvest. (See Table 3 for monthly moisture analysis beginning with storage period.)

The curves include the combined data for the three years, and are given separately for the two types of roots. From the date of entering storage in late October until the last analysis three months later, the mangels decreased in moisture content from 92.12 to 89.54 per cent. This amounts to an average loss of 2.80 per cent of the original moisture content. Similarly, rutabagas averaged 3 per cent loss of their original moisture content during the three months' storage period, the moisture content decreasing from 91.05 to 88.32 per cent.

In order to find at what point in the storage period the decrease in relative percentage of moisture was significant the data were tested statistically, P values being determined by the use of Fisher's (*4*) application of Student's method.

Because of the similarity in the rate of loss of moisture (fig. 1), mangels and rutabagas were grouped together and the significance of the decrease in moisture calculated for each of the three crop years. In 1925–26 the decrease in moisture was significant after two months of storage; in 1926–27 and 1927–28 the decreases were sufficiently pronounced to be significant after only one month of storage. In this connection it is well to emphasize that any decrease in percentage of moisture is a result of more rapid loss of moisture than of dry matter. If dry matter constituents happened to be reduced at the same rate as that at which moisture was lost there would be no change in percentage of moisture and dry matter present. Consequently it is probable that the actual loss was significant after still shorter periods than those determined.

It may also be noted from Figure 1 that during the month preceding harvest the roots increased in moisture content at approximately the same rate as that at which they declined during the first month of storage.

The moisture content of mangels was found to be approximately 1 per cent greater than that of rutabagas. This is in agreement with analyses given by Henry and Morrison (*5*).

CHANGES IN THE RELATIVE COMPOSITION OF THE DRY MATTER

Examination of the analyses on a dry-matter basis revealed only slight variations in the nutrients during the storage period. In testing the data for a measure of these changes the mangels and rutabagas were studied separately. Comparisons were made for each year's set of analyses, taking the data for the second and fifth periods of sam-

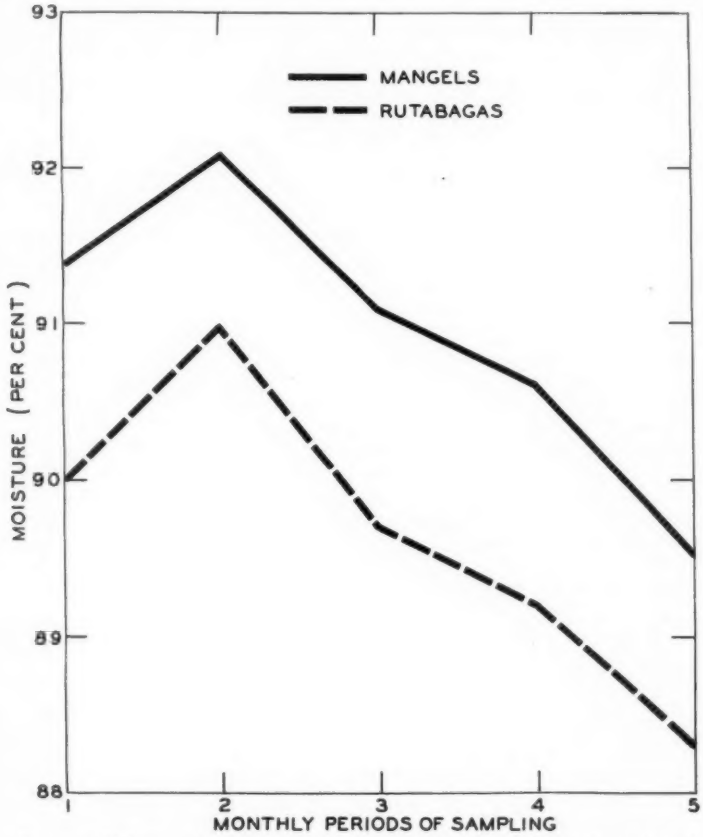


FIGURE 1.—Moisture content of mangels and of rutabagas from one month previous to harvest until three months after harvest. Average for all varieties for three years.

pling, representing three months of storage. Calculations for the mangels were based on 7 varieties for the season 1925–26, 6 varieties for the season 1926–27, and 8 varieties for the season 1927–28. The rutabaga comparisons included 5 varieties for each of the first two years and 4 varieties for 1927–28. As in the previous probability determinations, Fisher's (*4*) application of Student's method was used.

Changes in Nitrogen-Free Extract

Of the 7 varieties of mangels studied in 1925–26, 4 showed a loss and 3 showed a gain of nitrogen-free extract during the entire storage period of three months, resulting in an average loss of 1.55 per cent. The P value was 0.5460, indicating that the loss was not significant. The following season (1926–27) each of the mangel varieties showed a loss, the average for all varieties being 5.02 per cent of the nitrogen-free extract in the dry matter, which was significant as determined by a P value of less than 0.01. In 1927–28 each of the 8 varieties of mangels increased in nitrogen-free extract, the increases averaging 7.99 per cent, which is statistically significant. The effect of storage on the content of nitrogen-free extract in mangels has therefore been a significant loss for one year, a significant gain for another, and a non-significant change for the third.

With rutabagas, 12 of the 14 comparisons showed decreases in nitrogen-free extract during the 3-month period studied. In only one year (1925–26), however, was the loss great enough to be significant. The average decreases for the three separate years were 11.96, 4.31, and 3.82 per cent, respectively.

Changes in Crude Fiber

To test further the findings of previous investigators who, as mentioned before, believed that fermentative and respiratory changes cause appreciable losses during storage, the mangels and rutabagas were studied separately and the crude-fiber analyses at the time of storage were compared with those after three months of storage.

In 1925–26 and 1926–27 the crude-fiber content of the mangels showing an average increase each year of less than 1 per cent did not change significantly. In 1927–28, in which year an average decrease of 1.36 per cent of crude fiber was shown for each variety of mangels studied, the P value indicated that the decrease was definitely significant.

Without exception, there was an increase in the crude-fiber content of the dry matter of rutabagas during the three months of storage. The P values for the rutabaga study showed that these increases were definitely significant for the years 1925–26 and 1926–27, and probably significant for the year 1927–28, when the value was 0.0481. The respective average increases during storage for the three years were: 1925–26, 4.56 per cent; 1926–27, 2.33 per cent; 1927–28, 2.73 per cent.

Here the results are different for the two types of roots, there being a general increase in the crude-fiber content of dry matter with rutabagas and a decrease with mangels, and these notwithstanding the fact that both kinds of roots were stored under identical conditions in the same cellar.

Changes in Crude Protein

The study of the changes in the protein content of the dry matter during the 3-months storage period revealed results very similar to those obtained with crude fiber. With the mangels the comparisons were alike in that the P values for 1925–26 and 1926–27, representing small increases which average 1.20 per cent and 1.42 per cent, respectively, indicate that these increases were not significant, whereas in

1927–28 a loss of protein averaging 3.06 per cent for all varieties showed a change definitely significant when measured statistically. With the rutabagas P values for 1925–26 and 1926–27 show a significant increase in protein as an average for all varieties. For the year 1927–28 two varieties showed a slight increase and two a slight decrease in the protein content of the dry matter. The average change was not significant, as determined by a P value of 0.7999. The varietal increases in 1925–26 averaged 3.38 per cent and in 1926–27, 1.21 per cent; in 1927–28 the average change was less than 1 per cent.

RELATION BETWEEN DRY MATTER AND PROTEIN IN DRY MATTER

In studying the variation in the dry-matter content of the different varieties of roots included in the analyses, an interesting relationship was observed between the dry matter of mangels and the protein content of the dry matter.

The results of this comparison are shown in Table 1 and Figure 2.

TABLE 1.—*Relation between percentage dry matter and protein content of the dry matter of mangels*

[Comparisons given represent yearly average analyses of four periods of sampling for each variety studied during the respective years]

1925–26		1926–27		1927–28	
Dry matter	Protein in dry matter	Dry matter	Protein in matter	Dry matter	Protein in dry matter
7.54	17.55	6.07	18.95	7.85	11.86
7.99	15.09	6.35	15.17	8.12	10.39
8.10	15.67	6.53	19.84	8.95	10.37
10.22	13.25	7.32	16.24	9.35	8.55
11.48	11.47	8.22	16.16	9.60	8.90
11.77	9.77	8.92	15.80	10.90	7.44
12.44	10.23			11.22	7.92
				12.30	5.55

The figures in Table 1 are averages of the four analyses made during the storage of each variety of mangel. Observation alone would indicate an inverse relationship existing between the dry matter and the protein in the dry matter. The straight lines fitted to the data show, in Figure 2, that the relation was consistent for each of the three years.

This inverse relationship between the dry matter and the protein content of the dry matter does not hold for rutabagas. Table 2 gives the comparative analyses of rutabagas for the three separate years. Each analysis is recorded, instead of using the averages of four analyses as was done with the mangels (Table 1), in order to increase the number of the data for the purpose of measuring the relationship by means of the straight-line formula.

In Figure 3 is shown graphically the absence of any relation between the dry matter and the protein content of the dry matter of rutabagas. All three years gave similar values which are as consistent in failing to indicate a relationship as the corresponding values for mangels are in showing that such a relationship does exist.

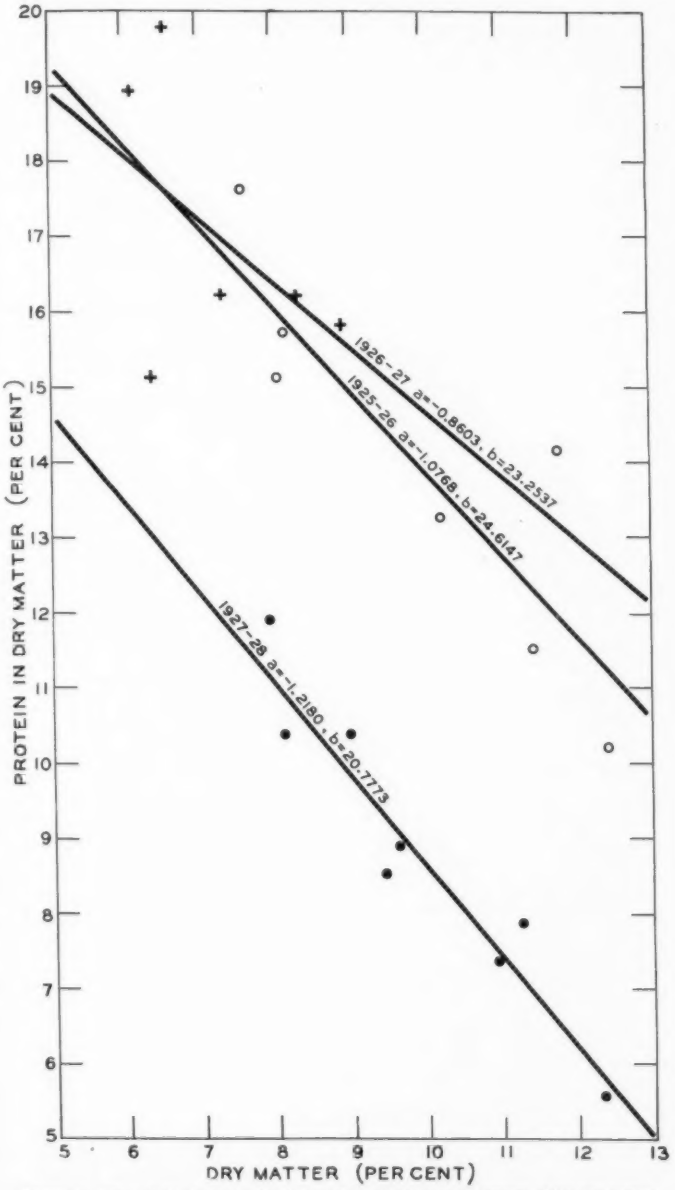


FIGURE 2.—The relation between the dry matter and the protein content of the dry matter of mangels. The lines passing through the observed values were obtained from the equation $y=ax+b$, in which y is the protein in the dry matter and x is the dry matter. Points on the graph representing values from the data of the three separate years are identified as follows: 1925–26 (○), 1926–27 (+), 1927–28 (●)

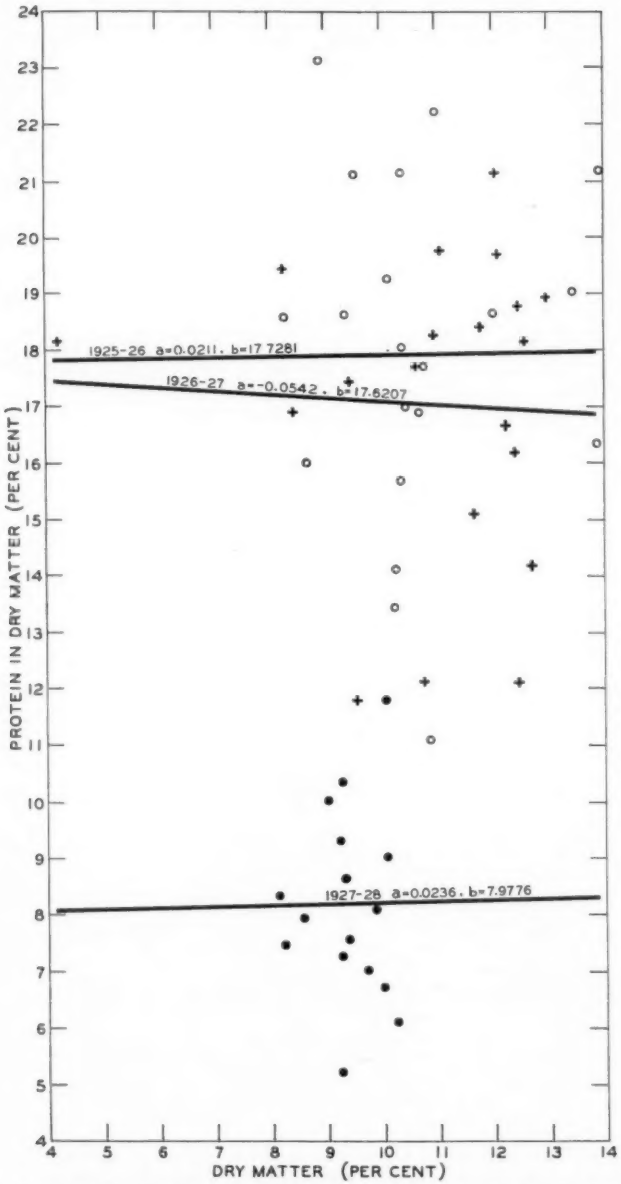


FIGURE 3.—The relation between the dry matter and the protein content of the dry matter of rutabagas. The straight lines passing through the observed values were obtained from the equation $y=ax+b$, in which y is the protein in the dry matter and x is the dry matter. Points on the graph representing values from the data of the three separate years are identified as follows: 1925-26 (○), 1926-27 (+), 1927-28 (●)

TABLE 2.—*Relation between the percentage dry matter and protein content of the dry matter of rutabagas*

Comparisons given represent analyses of each of the four periods of sampling for each variety studied during the respective years]

1925–26		1926–27		1927–28	
Dry matter	Protein in dry matter	Dry matter	Protein in dry matter	Dry matter	Protein in dry matter
8.36	18.66	4.20	18.17	8.20	8.32
8.72	15.96	8.30	19.45	8.30	7.52
8.96	23.12	8.50	16.85	8.60	7.91
9.42	18.59	9.50	17.43	9.10	10.04
9.55	21.16	9.60	11.76	9.30	9.34
10.12	19.20	10.70	17.70	9.30	10.35
10.26	13.45	10.80	12.66	9.30	5.26
10.29	14.10	11.00	18.20	9.36	7.33
10.36	15.72	11.10	19.69	9.40	7.65
10.39	21.14	11.70	15.07	9.40	8.65
10.39	18.00	11.80	18.44	9.80	7.04
10.49	16.99	12.10	19.67	9.90	8.17
10.70	16.82	12.10	21.16	10.00	6.69
10.82	17.74	12.30	16.60	10.10	9.01
10.91	11.07	12.40	16.07	10.10	11.84
11.08	22.19	12.50	18.82	10.30	6.06
12.06	18.62	12.50	12.16		
12.53	19.01	12.60	18.17		
13.88	16.32	12.70	14.16		
13.94	21.22	13.00	18.88		

Table 3 shows by period of sampling the combined 3-year average analyses of mangels and rutabagas during storage. The analyses given are on the basis of the original samples.

DISCUSSION

The data presented indicate that mangels and rutabagas undergo certain changes during storage, some of which are quite regular whereas others are irregular, depending no doubt upon both the physiological condition of the roots when stored and the conditions of storage.

The most noticeable as well as the most consistent change that occurred during storage was the continuous loss of moisture with the accompanying increase in dry matter. This was found in both types of roots and was sufficiently pronounced to be a factor of some importance in computing the feeding values of the roots after one or more months in storage.

A constant increase in moisture up to the time of harvest is somewhat at variance with the changes that take place in many maturing crops. With mangels and rutabagas it is evident that the physiological activities of growth continue at a rather rapid rate until the time of pulling. Changes due to maturity with decrease in moisture content as ordinarily thought of would probably not occur until the second season when seed is borne.

No data are available for the total losses in weight during storage. One might reasonably assume, however, that the total losses were due partly to loss of moisture as such and partly to loss of organic food substances respired during the storage period. If certain types of these food materials are used up more rapidly than others there will be corresponding changes in the relative composition of the dry matter

during the storage period. Table 4 gives the 3-year average content of crude protein, crude fiber, ether extract, and nitrogen-free extract in the dry matter of both types of roots at the beginning and at the end of the storage period. The ether extract may be practically disregarded as being present in such small amounts as to be relatively unimportant.

TABLE 3.—*Three-year average analyses of mangels and rutabagas (percentage) by periods of sampling*

Period of sampling No.	Moisture	Protein	Ether extract	Crude fiber	Ash	Nitrogen-free extract	Dry matter
MANGELS							
2	92.12	1.09	0.04	0.70	1.00	5.05	7.88
3	91.11	1.06	.06	.70	1.05	6.02	8.89
4	90.63	1.16	.09	.73	1.14	6.25	9.37
5	89.54	1.33	.09	.91	1.26	6.87	10.46
RUTABAGAS							
2	91.05	1.25	.08	1.12	.94	5.56	8.95
3	89.72	1.48	.10	1.42	1.18	6.10	10.28
4	89.21	1.64	.11	1.50	1.27	6.27	10.79
5	88.32	1.85	.09	1.79	1.52	6.43	11.68

The table shows that with rutabagas the greatest relative increase was in crude fiber, with a lesser increase in crude protein and a considerable decrease in nitrogen-free extract. This indicates that the losses of the rutabagas by respiration were heaviest in carbohydrate material (nitrogen-free extract). With mangels, however, the outcome was different. With these the greatest relative change in composition of dry matter during the storage period was a decrease in crude protein while the crude fiber remained practically constant and the nitrogen-free extract increased slightly. With mangels, then, the heaviest losses appear to be in nitrogenous material (crude protein). This apparent difference in type of food substance utilized during storage by two roots as closely related as mangels and rutabagas raises an interesting physiological question. Why should the one respire mostly carbohydrate material and the other mostly nitrogenous material? Further work on this point might prove to be of considerable interest.

From the practical viewpoint of the feeder it is clear that although the relative percentage of nutrient substances may increase during storage as a result of loss of moisture, nevertheless the total dry substance must be less after any given storage period. If this decrease in dry substance is due to loss of digestible and assimilable materials then the total feeding value of the crop is reduced by just that amount. On the other hand, if during storage considerable amounts of nonassimilable substances such as crude fiber were changed into more usable forms, there might conceivably be an actual gain in feeding value at the end of a suitable storage period. The results of this experiment indicate that the former condition existed in these trials since crude fiber either increased or remained approximately constant, while carbohydrates and nitrogenous material suffered the greatest reductions.

TABLE 4.—*Three-year average percentage of crude protein, crude fiber, ether extract, and nitrogen-free extract in the dry matter of mangels and rutabagas when stored, and after three months' storage*

Analyzed—	Crude protein	Crude fiber	Ether extract	N-free extract
MANGELS				
At beginning of storage period	13.83	8.88	0.51	64.1
After three months' storage	12.72	8.70	.86	65.7
RUTABAGAS				
At beginning of storage period	13.97	12.51	.89	62.1
After three months' storage	15.84	15.33	.77	55.1

A statistical examination by individual years of the changes in the various organic constituents during storage shows some of these changes sufficiently pronounced to be significant, yet they are not always consistent in any one direction. Attention has already been called to the fact that the percentage of nitrogen-free extract in the dry matter of mangels increased during one storage period and decreased during another, while the same constituent decreased one year in rutabagas, the changes in the other years being too slight to be significant. Likewise the percentage of crude fiber in the dry matter of mangels decreased significantly during one storage period, the other years being indeterminate, while with rutabagas the crude-fiber content increased significantly during two years and indicated a similar tendency during the third. A significant change of the protein in the dry matter of mangels, in the direction of a decrease in all varieties, occurred during the 1927–28 storage period; the changes for the two previous years were too slight to be significant. With rutabagas the opposite results were obtained, slight increases in the protein constituents for both of the first two years producing significant changes, whereas in 1927–28 the difference was not significant.

A marked difference was shown to exist between the mangels and the rutabagas in the relation between the dry matter and the protein content of the dry matter. Within the two different types of roots the relationship was very consistent for the three years studied. A high dry-matter analysis for mangels was indicative of a low protein content of that dry matter, and vice versa. With rutabagas the comparison showed very clearly that the variations as observed in the protein content of the dry matter were not influenced by the amount of dry matter found in the roots.

The existence of an inverse relationship in mangels may be partly explained on the basis of fertility and climatic conditions. Heavy applications of fertilizers, especially nitrogen, are conducive to luxuriant growth. Such growth is always of a more tender and succulent nature, and generally is richer in protein and lower in fiber. Why the rutabagas do not show this relationship also, when grown under conditions identical with those for the mangels, is not clear.

SUMMARY

When mangels and rutabagas were stored for a period of three months after harvest the loss of moisture was continuous and the rate of loss was fairly uniform. At the end of the storage period the dry-matter content of mangels had increased 2.58 per cent and that of rutabagas 2.73 per cent. This was equivalent to relative gains of 32.7 per cent for mangels and 30.5 per cent for rutabagas, respectively, and is of sufficient magnitude to be important in the calculation of feeding values.

In a study of the changes of the constituents of the dry matter during storage considerable variation was found between the two types of roots. Moreover, when the mangels and rutabagas were considered separately year-to-year variations were found to exist. As an average for the three years' work, the most pronounced changes in the relative composition of the dry matter of rutabagas was loss of nitrogen-free extract, and, to a lesser extent, of crude protein. With mangels, on the other hand, the greatest relative change appears to have been a loss in crude protein.

An inverse relationship was shown to exist between the dry matter of mangels and the protein content of the dry matter. With rutabagas there was no relation between these two constituents.

LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1925. official and tentative methods of analysis. compiled by the committee on editing methods of analysis. revised to july 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.

(2) Benoy, M. P.
1929. the respiration factor in the deterioration of fresh vegetables at room temperature. Jour. Agr. Research 39: 75–80, illus.

(3) Drummond, J. C., Channon, H. C., Coward, K. H., Golding, J., Mackintosh, J., and Zilva, S. S.
1924. the influence of the administration of certain oils on the nutritive value of the butter fat of cows on winter rations. Jour. Agr. Sci. [England] 14: [531]–547, illus.

(4) Fisher, R. A.
1928. statistical methods for research workers. Ed. 2, rev. and enl., 269 p., illus. Edinburgh and London.

(5) Henry, W. A., and Morrison, F. B.
1923. feeds and feeding; a handbook for the student and stockman. Ed. 18, 770 p., illus. Madison, Wis.

(6) Miles, W.
1841. on the cultivation of mangold-wurzel. Jour. Roy. Agr. Soc. England 2: 298–300.

(7) Miller, N. H. J.
1900–1902. experiments at rothamsted on the changes in the composition of mangels during storage. [I]–II. Jour. Roy. Agr. Soc. England (3) 61: 57–64, 1900; 63: 135–141, 1902.

(8) Pagnoul, A.
1891. über die zersetzung von aufbewahrten rüben. Zentbl. Agr. Chem. 20: 771–773.

(9) Shutt, F. T.
1902. on the changes in the composition of roots during storage. Canada Expt. Farms Rpt. 1901: 163–170.

10) Voelcker, A.
1859. the composition of a mangold-wurzel kept for two years. Jour. Roy. Soc. Agr. England 20: 131–134.

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